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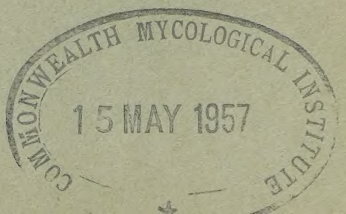

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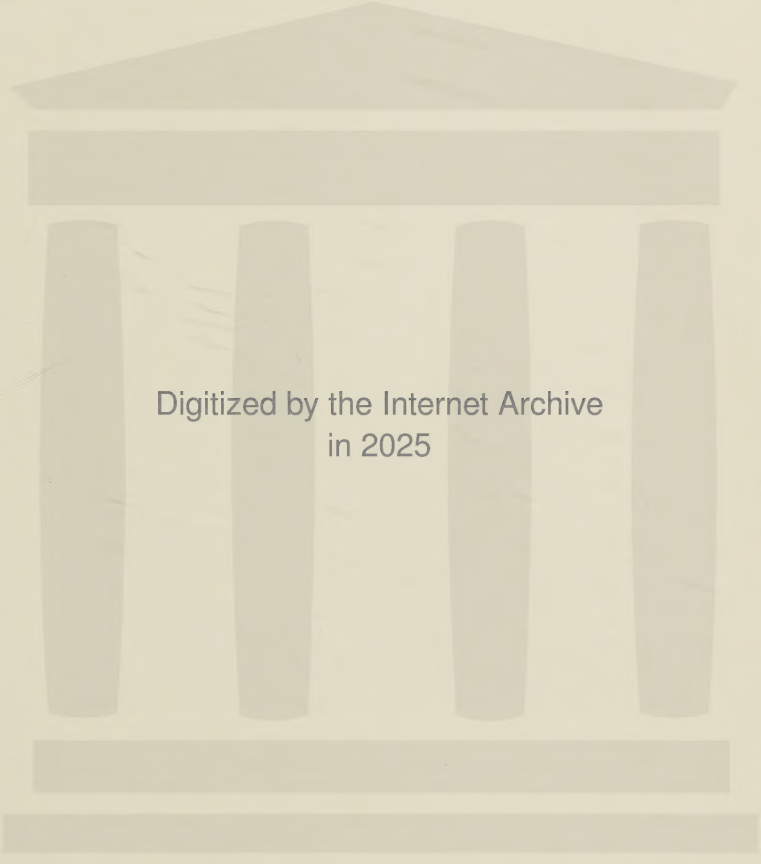

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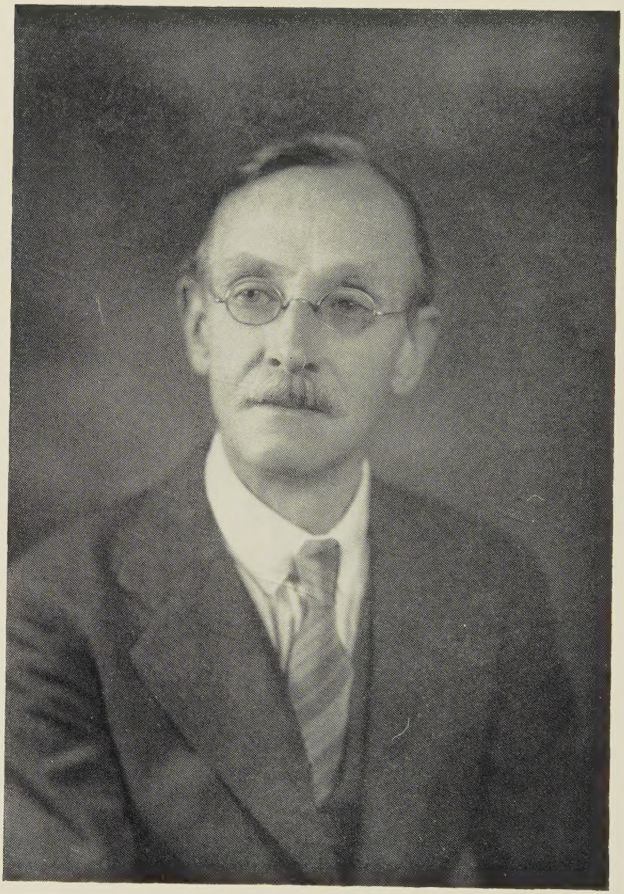

H. WORMALD

1879–1955

Trans. Brit. mycol. Soc. **39** (3), 289–290 (1956).

OBITUARY

HARRY WORMALD (1879–1955)

Harry Wormald, D.Sc., A.R.C.S., D.I.C., President of the British Mycological Society in 1940, Vice-President in 1941–2, Joint-Editor of the *Transactions* from 1931 to 1945, and a benefactor of the Society by a gift of books to the infant library in 1951, died in his home at Maidstone on 10 December 1955, at the age of 76.

He had retired from his post as Assistant-Director of the East Malling Research Station in 1939, but he edited the Annual Report of the Station until 1945, and continued his researches, collecting material for new editions of his monograph on the 'Brown Rot Diseases of Fruit Trees' (first published in 1935 as *Bulletin* no. 88 of the Ministry of Agriculture and Fisheries, and, after being completely rewritten, republished in 1954, as *Technical Bulletin* no. 3 of the same Ministry) and *Diseases of Fruits and Hops* (first edition, Crosby Lockwood and Son, Ltd., 1939, and by the same publishers in its final revised edition in 1955). These are the works on which his international reputation was built and on which it will rest. From 1945 to 1952 he worked daily at Bradbourne House, on the staff of the Commonwealth Bureau of Horticulture and Plantation Crops, preparing abstracts of phytopathological publications from all parts of the world, and of a wide range of papers on horticultural research in general. Wormald could read seven languages, including Russian, for, characteristic of the man, he had made it his business during his scientific career to learn to read the languages he needed for his work. Until 1949, when his health began to fail, he walked five miles daily to and from his work. Illness compelled him to retire from the Commonwealth Bureau in 1952. He then undertook the final revision of his two major works, and helped by Mrs Wormald and by his colleagues at East Malling, he completed that revision and saw the final editions in print, one in 1954, the other in 1955. Six months later he died. He had finished the work to which he had set his hand, and, beyond medical expectation, he had kept alive until the work was done.

Wormald became Joint-Editor of the *Transactions of the British Mycological Society* with John Ramsbottom and myself in 1931, and after Ramsbottom had retired from the editorial board, he continued with me until about the end of the war. At first, we met from time to time to deal with the affairs of the *Transactions*, but, so far as I recall, we did not meet after the war began, but carried on by correspondence. Wormald was a good colleague. He dealt thoroughly with manuscripts concerned with plant pathology, and he checked all the proofs, pathological and general, before they went for final printing. In this, as in all his work, he was quietly and effectively THERE. I never knew him to say or write anything unkind about anyone, and he never wrote or talked about himself. Even the occasional references to the war as it affected Kent, were restrained and objective. He had, as all editors have, some dislikes. He would not pass the word

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'considerable' for printing, he disliked 'in the case of' and its variations, and 'involved' also fretted him, for he said, rightly, that that word has become worn out by immoderate use. He would go to great trouble in looking out information with which to clarify obscurities in manuscripts. He had a searching and yet merciful pencil in dealing with verbosity. His careful editing must have saved the Society pounds in printing costs.

Wormald was born in Lofthouse, Yorkshire, where he attended the village school, afterwards going to the Lancaster Grammar School at Wakefield. He trained for a teacher at St John's College, York (1898–1900), and from 1900 to 1908 taught in Bradford and Leeds. He was by then a good field botanist, and he obtained a Board of Education Scholarship which took him to the Royal College of Science, Imperial College, where Prof. J. B. Farmer then had charge of the botany. In 1911 he took his Associateship (1st Class), and his B.Sc. (London). After the grant of the Marshall Scholarship and a beginning of research, leading to the award of the college diploma, he went to Wye College as assistant to Prof. E. S. Salmon in the Botany (Mycology) Department. Then really began his life work on plant pathology, and in particular his outstanding studies on the Brown Rot diseases of fruit trees. His M.Sc. came in 1916, and his D.Sc. in 1919. After that, a steady flow of publications on plant pathology and an ever-increasing influence on colleagues and students—Wormald became a recognized teacher of plant pathology in the University of London in 1932—continued to the end of his active life.

In 1923, Wormald, who had been working in association for some time with Dr R. G. Hatton, and so was already connected with the East Malling Research Station, went there as head of the newly organized section which is now the Plant Pathology Section. He went steadily on with his work on the pathology of fruit trees and hops, working with quiet tenacity, always content, once he had got to the heart of the matter, to leave to his colleagues the development of the practical applications.

Yet, for all his absorption in his scientific work, and for all the disinclination he had for anything which divided his attention, he did not allow everything else to drop. He was a good cricketer, a pianist of repute, a photographer, and, above all, a trusted and valued adviser to many seekers after scientific guidance. His field botany was not neglected; it gave him exercise as well as inspiration. Though he avoided distractions and all that they mean in the loss of time, he could not escape altogether. In addition to the work he did for the British Mycological Society, he was active for a time in the Association of Applied Biologists, and his office as Assistant-Director of the Station must have limited his time for research.

Quiet, patient tenacity, coupled with great unselfishness, devotion to his work, and indifference to fame: those were Wormald's characteristics. In his scientific work he would accept nothing less than the best attainable. He did not impose that iron discipline on his human associations. With people he was kindly and tolerant, looking for the good in them, encouraging honest endeavour. He showed, by example, doubtless quite unconsciously, that the good life comes to the man who forgets himself in his work.

I am greatly indebted to Dr R. V. Harris for many of the details of Wormald's career which I have set out in this tribute to a fine man. For the comments, I alone am responsible.

B. BARNES

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MORTIERELLA GLOBULIFERA ROSTRUP

By MYFANWY TURNER

Department of Botany, University of Nottingham

(With Plate 10 and 1 Text-figure)

Six cultures, isolated from soil or organic debris in soil, and assigned to *Mortierella globulifera* Rostrup (five with certainty and one tentatively) are described and the original description expanded. An experiment, the results of which suggest that this and other species of *Mortierella* are capable of producing proteolytic enzymes, is reported.

Introduction

Mortierella globulifera was described by Rostrup (1916), and few, if any, further observations have been published. The original description was very brief and did not mention the vegetative mycelium. Chlamydospores, stylospores and zygospores were not observed. As Rostrup described this fungus, it possessed tufts of simple sporangiophores, 0·5–1·0 mm. long, tapering from 20–28μ at the base to 4·5–5·5μ at the tip. Characteristic globose swellings were present at the base of the sporangiophores. The hyaline sporangia were spherical, 40–48μ, and contained globose spores, 6–7μ in diameter, with a strongly echinulate epispore. He illustrated a group of sporangiophores arising from a mass of rounded protuberances, sporangiophore apices with small collars, and round, echinulate spores. Rostrup considered that his species resembled *M. echinulata* Harz, which also has echinulate spores, and *M. tuberosa* van Tieghem and *M. pilulifera* van Tieghem which have rounded swellings associated with the base of the sporangiophore.

Origin of cultures examined

Four similar cultures of *Mortierella* were isolated on soil-extract agar from old pasture turf over Keuper marl soil. One of these (**60**) was isolated by the immersion tube method of Chesters (1948), two (**70** and **77**) from different samples of soil suspension, and one (**80**) from a fragment of decaying grass root. At first none of the cultures produced much aerial mycelium, and only a few simple sporangiophores. The spores were round and echinulate. In **77**, swollen protuberances were seen at the base of the sporangiophores, suggesting *M. globulifera*. The evidence for identification of the other three cultures was at first inadequate, but the similarity of vegetative habit, the numerous and characteristic chlamydospores, the simple sporangiophores and the echinulate spores suggested that all four were possibly the same species. They did not agree with the descriptions of any other known species, *M. echinula* Linneman (1953) and *M. traversiana* Peyronel (1913), with which they had some characters in common, being

ruled out because of the smaller, branching sporangiophores and the absence of basal swellings in these two species.

Later, cultures **60** and **80**, on potato-carrot agar at room temperature, began to produce aerial mycelium with numerous sporangiophores borne in clusters from aerial stolons. Two further cultures were received, one (**143**), isolated by Ward (1952) from a decaying pine needle, which produced aerial mycelium and sporangiophores in abundance and was readily identified as *M. globulifera*, and the other (**145**) isolated by Webster (1954) from a *Dactylis* root and already identified by him as *Mortierella globulifera*. The latter failed at first to produce aerial mycelium and sporangiophores, but closely resembled the other cultures and later produced numerous and characteristic sporangiophores. The six cultures were grown and compared on maize-extract agar, soil-extract agar, potato-dextrose agar, 2 % malt-extract agar, Czapek-Dox agar with 0·5 % yeast extract and on sterilized horse dung. Measurements of sporangiophores, sporangia and spores were made from unstained material mounted in 0·9 % saline.

Description of cultures

Vegetative mycelium. The submerged mycelium in all six cultures takes the form of a rosette of irregularly arranged, more or less pointed lobes usually about 5 mm. broad at the base, but often broader or narrower. The colony may grow evenly at first, but the lobed appearance is usually developed, at least near the edge of the dish. This character is reasonably constant on all agar media (Pl. 1, fig. 1). The lobed effect is due to repeated and close dichotomy of some of the principal hyphae, until the branchlets become so crowded that they cease to grow. Growth of the colony is carried on by other hyphae which originate near the base of a lobe, grow out without much branching at first, and later start to branch and form another lobe. The principal hyphae are slender, usually not more than 3–4 μ in diameter. The colony may consist solely of mycelium and prostrate surface hyphae, occupying the uppermost 1–2 mm. of the agar and appearing somewhat slimy. The environmental factors which influence the production of aerial mycelium and sporangiophores have not been determined. Varying conditions of temperature, aeration and nutrition do not seem to produce any consistent results, though on the whole media relatively poor in nutrients, e.g. soil-extract agar, seem more satisfactory than richer media, and temperatures about 18–20° C. more satisfactory than 24–28° C. Very little growth takes place at 30° C. and none at 35° C. When aerial mycelium is produced it forms a loose, cobweb-like network of aerial stolons, about 6 μ in diameter, attached to the substrate here and there by a few branching rhizoidal hyphae.

Chlamydospores are conspicuous in all cultures. They are formed abundantly in the submerged mycelium, but have not been seen in the aerial mycelium. They are usually intercalary, round to oval or somewhat irregular in outline, with smooth, rather thin walls and dense contents. Measurements for the six cultures, on potato-carrot agar, are given in Table 1. The nature of the medium does not affect the size or abundance of chlamydospores in any given culture.

Sporangiophores. The mode of origin of these structures is very characteristic, bearing some resemblance to that described and figured for *M. polycephala* Coemans by van Tieghem & Le Monnier (1873), Dauphin (1908) and Vuillemin (1918), for *M. reticulata* by van Tieghem & Le Monnier, and for *M. vantieghemi* by Bachmann (1900). It does not resemble the mode of origin of the sporangiophores of *M. tuberosa* van Tieghem and of *M. pilulifera* van Tieghem, since the swollen outgrowths at the base of the sporangiophore are themselves young (or possibly, sometimes, abortive)

Table 1. *Measurements of six isolations of* Mortierella globulifera *Rostrup*

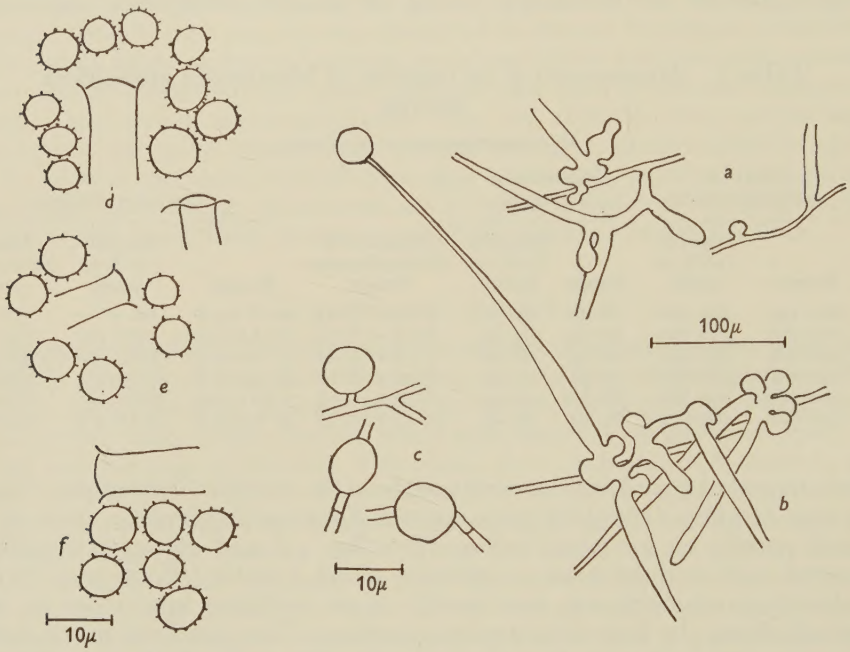
(All measurements are in microns.)

Culture no.	Length of sporangiophores		Diameter of sporangia		Diameter of chlamydospores	Diameter of spores		
	Range	Majority (50% or more)	Range	Majority (50% or more)	Range	Range	Majority (50% or more)	Mean of 100 measurements
60	260–450	300–400	18–42	30–40	6–14 × 6–13	4– 8 × 4–8	6·0 × 6·0	6·1 × 5·9
70	200–750	400–600	20–54	35–45	6–14 × 6–14	5–11 × 4–9	7·0 × 7·0	7·3 × 6·8
77	200–400	280–320	20–44	26–30	7–12 × 7–11	4– 7 × 3–7	5–6 × 5–6	5·6 × 5·4
80	150–1080	400–600	19–54	30–45	8–12 × 8–12	4– 9 × 4–8	6–7 × 6–7	6·6 × 6·0
143	220–1800	400–600	18–50	30–45	10–13 × 10–13	5–11 × 4–9	6–8 × 6–7	6·8 × 6·5
145	250–600	400–600	20–54	30–45	10–16 × 10–14	4– 9 × 4–8	5–7 × 5–7	6·2 × 5·9

sporangiophores and are not comparable to the nutritive vesicles described by van Tieghem (1875) for these species. Each sporangiophore arises as a small nodule on an aerial stolon (Text-fig. 1*a*) and elongates rapidly. Several such nodules arise at intervals along a stolon. Some may form isolated sporangiophores, but usually more swellings arise close to, or actually from, the base of each sporangiophore. These grow out into finger-like processes and may branch, appearing like inflated rhizoids (Text-fig. 1*b*). Some may perhaps act as rhizoids, but most develop into sporangiophores, which thus appear in tufts, with further swellings arising at the base of each tuft. The sporangiophores are unbranched, from 150 to 1800 μ long, the majority being 300–600 μ. The axis is inflated at the base (usually above a slight constriction) and tapers smoothly to the apex. The largest sporangiophore seen was 18 μ broad at the base and 9 μ at the tip, but the majority are 10–12 μ at the base and 4–5 μ at the tip. No sporangiophores quite as stout as those mentioned by Rostrup were seen. Cultures on horse dung do not differ in this respect from those on agar media, nor do five of the six cultures differ appreciably from each other, though the sporangiophores of **77** are shorter than those of the other cultures (Table 1).

Sporangia and spores. Sporangia of *Mortierella* species are difficult to measure, owing to the ready dissolution of the very delicate wall. The measurements quoted are of sporangia sufficiently mature to show separation of the spores, but still immature enough to be mounted intact. The sporangia are hyaline and at first perfectly round in outline, but later the delicate wall moulds itself to the outline of the spore mass. On dissolution it leaves a small but distinct collar. The transverse septum is very slightly

convex. Usually each sporangium contains about twenty spores, but larger or smaller sporangia may contain more or fewer spores. The spores tend to adhere together in masses after dehiscence of the sporangium. They are strongly echinulate, and round to slightly oval, with occasional irregularly shaped individuals due to incomplete separation. Measurements for the six cultures are given in Table 1.



Text-fig 1. *M. globulifera* Rostrup. (*a*) Origin of sporangiophores from aerial stolon, culture **80**; (*b*) group of mature and young sporangiophores, culture **60**; (*d–f*) sporangiophore apices and spores, cultures **145**, **143** and **80**; (*c*) chlamydospores, culture **143**.

Nutrition

No thorough nutritional study of these cultures has been made, but all the isolations of *M. globulifera*, and cultures of other *Mortierella* species including *M. bainieri* Costantin, *M. hygrophila* Linneman, *M. isabellina* Oudemans and *M. vinacea* Dixon-Stewart were grown on starch agar (plain water agar with 1 % starch), cellulose agar (water agar with 2 % bacterial cellulose prepared from washed membranes of *Acetobacter xylinum*), milk agar (water agar with 5 % sterile milk) and gelatin agar (water agar with 4 % gelatin). Growth occurred on all these media, and on water agar without additions, but was most luxuriant on the milk agar, as might be expected. None of the cultures showed any sign of cellulose decomposition, whereas a culture of *Trichoderma viride* used as a control produced some clearing of the slightly opaque medium. On flooding the starch plates with iodine, only *Mortierella isabellina* and *M. vinacea*, of the *Mortierella* species tested, gave a

very slight indication of hydrolysis. On milk agar all the *Mortierella* cultures showed complete clearing of the medium under the colony and narrow but distinct zones of clearing beyond the margin of the colony. The greater breadth of the clear zone at some points suggests that variation in this respect may occur between sectors of the same colony (Pl. 1, fig. 2). Pieces of agar, bearing mycelium, were cut out, placed on fresh plates of milk agar and incubated at 35° C. No growth occurs at this temperature, but the substance responsible for the clearing of the medium diffused out and produced clear patches in the new medium. On gelatin agar growth was slower, probably owing to the low carbon-nitrogen ratio. After treatment with acid mercuric chloride, the medium below the colonies appeared clear, indicating hydrolysis of the gelatin, but the clear area did not usually extend beyond the margin of the colony (Pl. 1, figs. 3, 4). Cultures of *Mucor racemosus* and *M. pusillus* on the same media showed no sign of proteolytic activity, whereas a culture of *Thamnidium elegans* showed marked activity.

While this experiment does not provide sufficient data for any general conclusion, the results agree with Bachmann's findings for *M. vantieghemi*, and with the fact that species of *Mortierella* are frequently found upon natural substrates rich in organic nitrogen, and suggest that some species, at least, are capable of producing proteolytic enzymes.

Conclusion

Five of the six cultures are clearly referable to *Mortierella globulifera* Rostrup. Culture **70** has so far produced only isolated sporangiophores, without the characteristic basal swellings, but the remaining characters so strongly resemble those of the other five cultures that it is probably the same species. Rostrup's original description may accordingly be amplified.

M. globulifera Rostrup. Substrate mycelium on agar media usually forming a rosette of more or less pointed lobes. Chlamydospores abundant in substrate mycelium, round to oval, mostly 8–10 μ in diameter. Aerial mycelium sparse, cobweb-like, consisting of stolons from which the sporangiophores arise, at first singly. Young sporangiophores appearing as swollen outgrowths around the bases of older sporangiophores, and forming tufts. Sporangiophores simple, 150–1800 μ long (mostly 300–600 μ), axis 6–18 μ broad at the base (according to Rostrup 20–28 μ) but often slightly constricted below the broadest part, tapering to about half the maximum breadth at the apex. Sporangia round, hyaline, mostly 30–45 μ in diameter (according to Rostrup 40–48 μ). Wall readily diffluent, leaving a small collar. Spores round to oval, echinulate, mostly 5·5–7·0 μ in diameter. Stylospores and zygospores not observed.

The writer wishes to thank Dr G. M. Ward and Dr J. Webster for the two cultures mentioned above, and Prof. C. G. C. Chesters for his kind advice and help, also Mr D. Allen and Mr R. Johnson for the photographs.

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EXPLANATION OF PLATE 10

Mortierella globulifera

Fig. 1. Culture **143** on potato-dextrose agar, 7 days.
Fig. 2. Culture **145** on milk agar, 7 days.
Fig. 3. Culture **70**. Fig. 4. Culture **143**. Both on gelatin agar, treated with acid mercuric chloride after 5 days.

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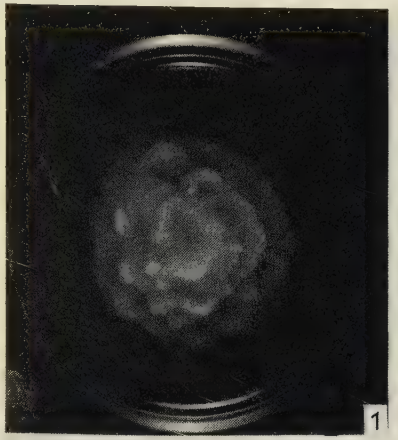

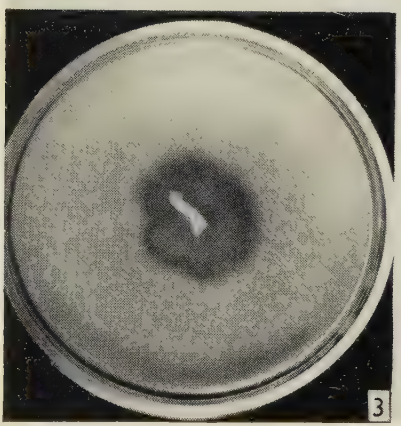

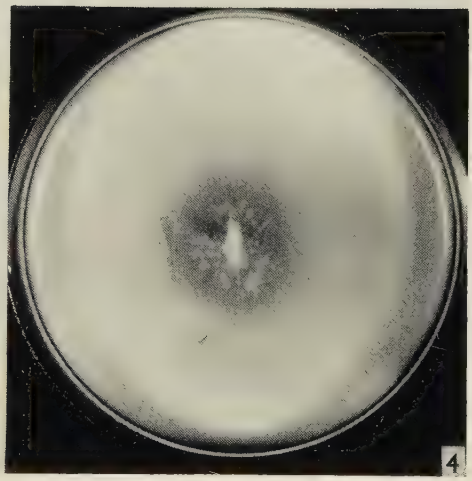

(*Facing p.* 296)

Trans. Brit. mycol. Soc. **39** (3), 297–300 (1956).

NECROTIC SPOTTING OF THE OIL PALM BY *CERCOSPORA ELAEIDIS* STEYAERT

By W. G. KOVACHICH

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(With 2 Text-figures)

A foliar disease of adult oil palms (*Elaeis guineensis*) is described. It is demonstrated that the causal organism, *Cercospora elaeidis* Steyaert, is a different strain of the fungus from that responsible for a leaf spotting of nursery oil palms. As *Oplothecium arecae* Sydow is closely associated with the disease spots, the morphology of this fungus is briefly described.

Occurrence and symptoms

An illustration of an oil palm leaflet showing Necrotic Spotting was given by Moureau (Vanderweyen, 1952), who considered that the condition was caused by microscopical insects feeding on the mesophyll cells. More recently, Bull (1954) described and illustrated the disease, which he suggested was probably fungal in origin.

Necrotic Spotting is probably the most common foliar disease of adult, plantation oil palms. The degree of infection varies in different plantation blocks and also between individual palms, but it has not been possible to determine any relationship between the degree of infection and any specific, environmental condition. Similarly, no differences have been observed in the incidence of the disease, following the application of various fertilizers. Palms of three years and older are normally infected, and although nursery palms are susceptible, it is rare amongst such palms. No information is available concerning the effect of Necrotic Spotting on fruit production, and it is generally considered to be of only minor economic importance and unworthy of an expensive control programme.

Infection may occur on the leaflets of the third or fourth whorl of leaves but the disease is, usually, only severe in the two lower whorls. The most typical, early symptoms of infection are seen on the under-surface of the leaflets and take the form of pin-point, brown spots surrounded by a small, superficially water-soaked area. This area enlarges irregularly along the interveinal tissue, and is followed by the enlargement of the central brown spot. The water-soaked area then becomes yellow and, later, orange coloured. When the spot is fully developed it consists of a round- to oval-shaped brown area 3–4 mm. in diameter surrounded by an irregular, orange 'halo' which is oval-shaped and measures, approximately, 10 × 4 mm. Desiccation of the spot and 'halo' may occur, and it is not unusual for adjacent spots to coalesce and for heavily infected parts of the leaflet lamina to dry out completely.

When the under-surface of fully developed spots is examined with a low-

power binocular microscope, conidiophores and conidia of a species of *Cercospora* may be seen arising from the centre of some of the spots. Small black perithecia of *Oplothecium arecae* may also be frequently observed scattered over the central part of spots which are beginning to desiccate.

The causal organism

Proof of pathogenicity. Cultures from diseased leaflets resulted in the isolation of a species of *Cercospora* resembling that associated with the diseased spots. A spore suspension in sterile water was prepared from these cultures and painted on the under-surfaces of three leaflets on each of three leaves of 3-year-old palms. A further three leaflets on each of these leaves were painted with sterile water as a control. The first symptoms of Necrotic Spotting were observed on the inoculated leaves 18 days after treatment, and the fully developed spots were apparent 32 days after inoculation. The control leaflets remained uninfected. The inoculated fungus was re-isolated from the infected areas of the inoculated leaflets.

Identification of the causal organism. A culture of the *Cercospora* used for the inoculation experiment was sent to the Commonwealth Mycological Institute, Kew, where Dr M. B. Ellis considered the fungus morphologically indistinguishable from *C. elaeidis* Steyaert., both on the host and in culture. It has already been shown that *C. elaeidis* is the causal organism of a disease of nursery oil palms (Kovachich, 1954). As different symptoms are exhibited by the two diseases, it was decided to test whether the different symptoms were due to two different strains of the fungus or to the age of the palm infected. Young, five-leaved seedlings were inoculated with isolates from the two diseases. Symptoms of both diseases were reproduced on these seedlings and it is, therefore, considered that there are two different strains of *C. elaeidis*, which are pathogenic to the oil palm. This view is supported by the presence of both types of spotting on leaflets of some 3-year-old palms.

Morphology of C. elaeidis. The conidia of the Necrotic Spotting strain are practically identical with those of the strain responsible for the nursery disease. They are hyaline to slightly olivaceous, 3–9 septate, obclavate with a well-defined basal scar, 75–160 μ long and 6–7 μ wide at the widest point. The conidiophores measure 140–410 μ long with an average of 210 μ compared with an average length of 145 μ for the nursery strain. The conidiophores occur in groups of 3–12, joined in hyphal knots at the mouth of the stomata (Fig. 1).

C. elaeidis is more easily isolated from necrotic spots than from the nursery disease condition. However, cultures of the two strains on all the media employed have been very similar.

Oplothecium arecae Sydow

On old disease spots, the perithecia of *Oplothecium arecae* are superficial, black, spherical, brittle with short, stiff appendages, which are forked and pointed. No ostiole could be observed prior to the liberation of the spores, and it is considered that the perithecium dehisces by an irregular rupture

of the apex. The perithecia measure 60–90 μ in diameter, whilst the appendages are 12–15 μ long. The external mycelium is hyaline and arises from the stomata of the host. Numerous asci are present within the

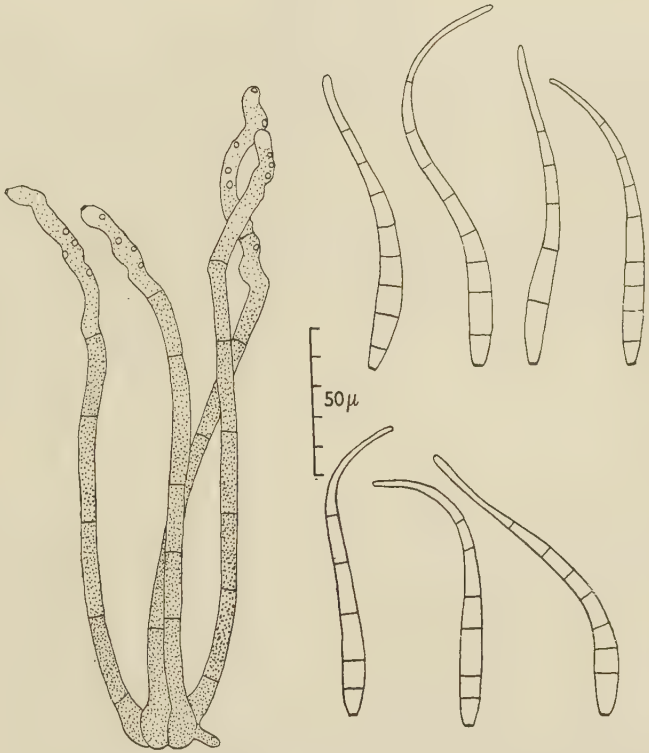

Fig. 1. Conidiophores and conidia of *C. elaeidis* isolated from necrotic spots.

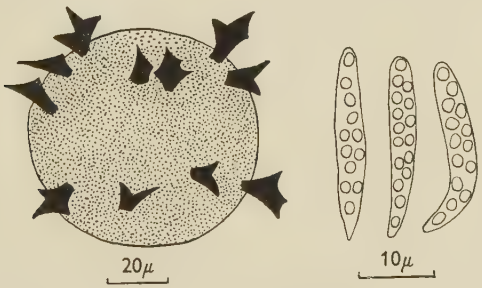

Fig. 2. Perithecium and asci of *Oplothecium arecae* Sydow.

unilocular perithecia but paraphyses are absent. The asci measure 17–26 μ long and 3–5 μ wide, are cylindrical to clavate and contain 12–16 hyaline, single-celled ascospores which are, approximately. 2·5 μ in diameter (Fig. 2).

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A COMPARISON OF TECHNIQUES FOR ISOLATING SOIL FUNGI

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Parallel studies were made with different techniques for isolating fungi from soil. Isolations by screened immersion plates showed close agreement with those by more direct methods (immersion tubes and direct inoculation) and differed from those by dilution plates and soil plates. The latter methods favour sporulating species and results may be further biased by using differentially selective media. Isolations by screened plates exhibited a wider range and variety of species than those obtained by any other method, particularly for species of *Mortierella* and dematiaceous fungi, and also provided a quantitative measure of the relative distribution of species.

A modification of the Rossi-Cholodny slide technique made possible the isolation of fungus mycelium from the slides.

Introduction

Extensive publications concerning soil micro-fungi mainly relate (Chesters, 1949) to the number and species of fungi, to the ecological factors influencing the soil micro-population, to growth relations between micro-organisms and higher plants, and to the chemistry of the breakdown of plant remains by fungi, in soil and *in vitro*.

Too often investigations of the fungus ecology of soil fail to differentiate between dormant and active phases. Since fungal activity depends upon the differing food sources and the ever-changing environment it has been thought more appropriate to assess the fungus potentiality of a soil, because, as the various and characteristic conditions present themselves, so will the diverse components of this microflora become active. Investigations have usually reported the mass isolation of many fungi from soil by diverse techniques, and few attempts have been made to correlate this floristic information with field conditions.

It appears to us essential to distinguish between the active and the dormant states of fungi in soil, since it is as physiologically active, vegetative mycelium that fungi play their part in the decomposition and transformation of plant and animal residues which result in the release of mineral nutrients essential for higher plant growth, and in the aggregation of soil particles. We believe that in a particular soil, at any one time, there is a characteristic fungus flora in an active mycelial state which will vary with such factors as the precise soil profile and the vegetation it carries, soil moisture and temperature, the season and the stage of decomposition of organic residues. By characterizing the active micro-flora of a soil under changing environmental conditions, it may be possible to correlate seral stages in the soil population with such changes. In this

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manner a more realistic and useful contribution to the ecology of fungi in soil can be achieved.

The present investigation embraced the development of the screened immersion plate method (Thornton, 1952) for isolating soil fungi and its application to assess the relative abundance of species occurring in soils derived from Bunter Sandstone in north Nottinghamshire. This paper presents the results of comparative trials with the screened immersion plate and with other soil microbiological techniques. A later contribution will report an investigation of the fungi occurring in Bunter soils, employing the screened immersion plate method.

Comparison of techniques

The screened plate technique was used under field conditions in comparative trials with the following direct and indirect techniques: immersion tubes (Chesters, 1948), direct inoculation (Waksman, 1916), a modified Rossi-Cholodny slide technique (devised by R. H. Thornton), dilution plates, and soil plates (Warcup, 1950).

(1) *Screened immersion plate: immersion tube*

The immersion tube method, designed and applied by Chesters (1940, 1948) for the isolation of fungi from soil, and the screened immersion plate technique (Thornton, 1952) share two features in common. Both methods attempt the isolation of mycelium from soil, and both techniques introduce an agar medium into natural soil so that the medium is exposed to soil only at selected sites.

The isolating medium was 2 % water agar and the growth which developed was transferred to pure culture on potato-dextrose agar for subsequent identification. Seventeen immersion tubes and ten screened immersion plates (i.e. 102 capillary orifices and 100 screen apertures respectively) were buried for 14 days (2–16 March 1950) in the A horizon of a brown forest soil under species of *Pinus* at ten sites at 2 yd. intervals in a 20 yd. transect.

Soil conditions. Moisture (dry-weight basis) 6·5–8·0 %; temperature, 4–7° C.; reaction (glass electrode), pH 4·8.

Results are presented in Table 1 and express the isolation frequency of each species as a percentage of the total number of isolations by each method.

Both methods showed a similarity in the number of apertures and orifices exhibiting growth and in the total number of isolations effected. Fewer species were isolated by the immersion tubes. Colonization of immersion tubes by fungi depends upon the ability of individuals to compete successfully with other members of the soil population for entry through capillary orifices. Also, once established the fungi must be capable of growing into the depth of the medium far enough to be isolated in the agar core. This implies tolerance of the low oxygen tension resulting from sterilization of the agar during preparation. The predominance of *Mortierella humilis* Linm. and *Mucor hiemalis* Wehm. in immersion tubes is only partially

confirmed by the screened immersion plate method. Since these two species are normally characterized by strong and rapid growth on agar media it is possible that, once established, they would be likely to colonize the bulk of the agar within the tubes and would therefore be recorded with

Table 1. *A comparison of fungi isolated by screened immersion plates and by immersion tubes*

Species	Percentage frequency of isolation (approx.)	
	Screened immersion plates	Immersion tubes
Mucor hiemalis Wehm.	2	16
Mortierella alpina Peyr.	2	—
M. gracilis Linm.	6	8
M. humilis Linm.	20	54
M. jenkini (Smith) Naumov	4	—
M. minutissima van Tiegh.	4	—
M. vinacea Dix.–Stew.	23·5	8
Yeasts	2	—
Pseudogymnoascus roseus Raillo	2	—
Phoma sp.	4	—
Streptomyces sp.	2	—
Penicillium cyaneo-fulvum Biourge	2	2
P. janthinellum Biourge	—	4
P. melinii Thom.	—	2
Sporotrichum sp.	2	—
Tricothecium roseum Link	—	2
Botrytis cinerea Fr.	2	—
Cylindrocarpon didymum (Hartung) Wollenw.	2	—
Humicola grisea Traaen	6	2
Cladosporium herbarum (Pers.) Link	2	—
Sterile mycelium	14	2

a high frequency of isolation. The excretion of metabolic products by such colonizing organisms might also prevent or retard the establishment of other species and thereby prevent their isolation. Evidence for this view was provided by Nicot & Chevaugeon (1949), who showed that the predominance of *Acrostalagmus koningi* (Oud.) Duché et Heim within tubes from a particular soil may have been associated with the ability of that organism to produce, in culture plates, a substance antibiotic toward additional organisms isolated from that soil when plating methods were applied. Chesters (1948) recognized that such a control of isolated fungi may be effected at this point.

With screened immersion plates the larger, 0·5 cm. diameter, aperture may tend to lessen the competition for entry, while the use of thin agar films guards against low oxygen tensions in the agar. The spacing of screen apertures and the regulation of incubation by means of control plates prevented growth under one aperture from influencing the growth under an adjacent aperture.

The microscopical examination of immersion plates during isolation enabled a rapid, clear and direct determination of the presence or absence of mycelium. Although a rough examination of immersion tubes could be made with a hand-lens prior to sampling, it was generally necessary to sample each tube and incubate agar sections to demonstrate the

presence of mycelium. The preparation, insertion and sampling in both methods utilized a similar amount of time and labour. Chesters (1948) found that the use of differently enriched media within tubes variously influenced the type and frequency of isolation of different genera and species.

(2) *Screened immersion plate: direct inoculation*

Early realization of the limitations of the dilution plate method led Waksman (1916) to develop a method of direct inoculation. Mycelium developing within 24 hr. from soil particles inoculated on to agar media was believed to have come from mycelium in soil, because parallel studies showed that growth from spores could not have developed a similar bulk of mycelium. Waksman's direct inoculation method and the screened immersion plate technique were therefore compared. Particles of soil, taken from composite soil samples, were inoculated directly on to the surface of potato-dextrose agar in Petri dishes. After incubation for 24 hr. at 24·5° C., culture plates were reversed and viewed under a binocular dissecting microscope (×25). Particles exhibiting fungus mycelium were identified by an ink mark on the base of the Petri dish, and incubation was then continued for a further 18 hr. before transferring inocula to pure culture for subsequent identification.

Six agar plates were inoculated from each of the three horizons of a brown forest soil under oakwood. Five screened immersion plates were buried in each of the three horizons for 14 days during October 1950.

Soil conditions:

Horizon	Moisture, % (D.W. basis)	Temperature (° C.)	pH	Organic carbon (Walkley & Black values)
A	15·5	10	5·1	0·89
B	16·3	9·8	5·0	0·74
C	8·0	9·7	5·0	0·04

Results are set out in Table 2.

Table 2. *A comparison of fungi isolated by screened immersion plates and by the direct inoculation method*

Species	Screened immersion plates	Direct inoculation method
Zygorhynchus moelleri Vuill.	—	*
Mortierella acuminata Linm.	*	—
M. gracilis Linm.	*	—
M. humilis Linm.	*	*
M. nana Linm.	—	*
M. vinacea Dix.–Stew.	*	—
Fusidium sp.	*	—
Cephalosporium acremonium Corda	*	—
Trichoderma viride Fr.	*	*
Penicillium frequentans Westl.	*	*
P. notatum Westl.	*	—
P. purpurescens (Sopp.) Thom & Raper	*	—
Sporotrichum sp.	*	—
Cylindrocarpon didymum (Hartung) Wollenw.	—	*
Trichocladium opacum (Corda) Hughes	*	—
Bisporomyces chlamydosporis van Beyma	*	—
Sterile brown mycelium	*	—

The short incubation period employed with the soil particle technique favours not only those organisms which grow more rapidly on enriched agar, but also those which can best utilize the particular nutrients employed. Such species will overgrow more slowly developing species and prevent their isolation, while the possible excretion of metabolic products may inhibit the development of additional species. Jensen (1931) found that the direct method isolated spreading rather than localized mycelium, and the fungi isolated in this experiment may well belong to the former group.

(3) *Screened immersion plate: modified Rossi-Cholodny buried slide technique*

The independent development by Rossi (1928) and Cholodny (1930) of a buried slide technique advanced the study of micro-organisms *in situ*, and provided an impetus to soil microbiological work. Conditions around buried slides were realized to be unnatural because the smooth, even glass surface presents conditions absent in the irregular, discontinuous habitats of the soil; also, moisture tends to accumulate on such surfaces. Despite these features, the method has provided a fresh outlook on microbial conditions in soil (Demeter & Mossel, 1933; Blair, 1943).

Applied to the study of fungi, this technique was considered by Conn (1932*a*, *b*) and by Jensen (1934, 1935) to give more reliable information regarding the activity of mycelium in soils than dilution plating methods, though the lack of qualitative data (Demeter & Mossel, 1933) is a disadvantage. Isakova (1938) offered a solution to this problem by overpouring previously buried, yet unstained, slides with melted and cooled agar media. Growth which occurred after a period of incubation was isolated and identified. Isakova was able to supplement, with qualitative data, quantitative information obtained from normal stained slides. A similar technique was applied by Ziemiecka (1935), in which various nutrient solutions were dried on slides and their effect on the soil population assessed after a period of incubation in soil.

Method. Clean and sterile 3 × 1 in. microscope slides were pressed against freshly exposed and evenly trimmed soil faces within selected soil profiles, and held in place by a U-shaped glass rod. Excavated soil was replaced in its natural layers and the slides left for a period of incubation. After removal, slides were gently tapped to remove adhering soil clumps and placed, exposed face downwards, on 30–40 ml. Czapek agar + 2 % yeast extract in sterile Petri dishes. The previously exposed surfaces were then viewed under a binocular microscope (× 25), through the back of the slides. Any mycelial growth was marked by ink on the upper surface of the slides. Dishes were incubated for 43 hr. at 24·5° C. The volume of agar beneath each slide was cut out with a sterile scalpel and the slide and adhering agar transferred to the apparatus employed for sampling screened agar films. Agar inocula containing hyphae were cut out with a sterile needle and transferred to pure culture for subsequent identification.

Five slides and five screened immersion plates were buried in each of three horizons of a brown forest soil under oakwood, and in each of four horizons of a podzol soil under *Calluna* heath, for 14 days during October 1950. Results are presented in Table 3 and give the species isolated from

the various soil horizons by the modified Rossi-Cholodny and the screened plate techniques.

Table 3. *A comparison of fungi isolated by screened immersion plates and by a modified Rossi-Cholodny buried slide technique*

Species	Screened immersion plates		Modified Rossi-Cholodny technique	
	Brown forest	Podzol	Brown forest	Podzol
Mucor hiemalis Wehmer	—	—	*	—
M. ramannianus Moeller	—	*	—	*
Zygorhynchus moelleri Vuill.	—	*	*	*
Mortierella acuminata Linm.	*	—	—	—
M. exigue Linm.	—	*	—	—
M. gracilis Linm.	*	*	—	*
M. humilis Linm.	*	*	*	*
M. nana Linm.	—	*	*	—
M. stylospora Dix.–Stew.	—	*	—	—
M. vinacea Dix.–Stew.	*	*	—	—
Yeasts	—	*	*	—
Fusidium sp.	*	—	—	—
Cephalosporium acremonium Corda	*	*	*	*
Cephalosporium sp.	—	*	—	—
Trichoderma viride Fr.	*	*	*	*
Penicillium frequentans Westl.	*	*	—	*
P. implicatum Biourge	—	*	—	—
P. lividum Westl.	—	—	—	*
P. melinii Thom	—	*	—	—
P. notatum Westl.	*	*	—	—
P. purpurescens (Sopp) Thom & Raper	*	—	—	—
P. spinulosum Thom	—	*	*	*
P. stoloniferum Thom	—	*	—	—
Botrytis cinerea Fr.	—	—	*	—
Botrytis sp.	—	*	—	—
? *Torula* sp.	—	*	—	—
Bisporomyces chlamydosporis van Beyma	—	*	—	—
Hormodendron cladosporioides (Fres.) Sacc.	—	*	—	—
Cylindrocarpon didymum (Hartung) Wollenw.	*	—	—	—
Sterile brown mycelium	—	*	—	—

In general, the fungi isolated from Rossi-Cholodny slides were similar to the more numerous fungi isolated by screened immersion plates. The flora isolated by the slides may be influenced by the composition of the agar medium employed, and by the ability of species to grow into the agar under conditions of reduced oxygen tension. It would be an improvement in future work to use thin films of agar in the Petri dishes, because agar blocks can be cut with greater precision from such films and this is important when isolating adjacent mycelial growths.

(4) *Screened immersion plate: dilution plate*

Much of the information concerning fungi in soil has been gained by the dilution plate method. Waksman (1922) and Jensen (1931) adjusted the isolation medium to *c.* pH 4·0 by sulphuric acid to reduce the growth of bacteria and of Actinomycetes. Several common soil fungi, however, fail to develop on plates rendered sufficiently acid to prevent the growth of bacteria and Actinomycetes. Tyner (1944) suggested the use of boric acid in place of sulphuric acid. Smith & Dawson (1944) proposed the use of rose

bengal at a concentration of 1:15,000 as a bacteriostatic agent, which Dawson & Dawson (1947) found to produce no fungistatic effect other than a reduction of colony size. Martin (1950) recommended the use of a peptone-dextrose agar containing 1:30,000 rose bengal and 30 μg. streptomycin. A peptone-dextrose medium containing 1:15,000 rose bengal was slightly better than a glucose-nitrate-soil extract agar containing a similar concentration of rose bengal, and both methods were significantly better than an acidified agar medium as regards numbers and types of organisms isolated.

For the present study, dilutions of soil in sterile tap water at concentrations of *c.* 1/5000 and 1/100,000 were chosen, since preliminary trials gave thirty to fifty fungal colonies per plate at the lower dilution. This number was sufficient for quantitative estimations (James & Sutherland, 1939) and permitted the isolation of the various species. A higher dilution was included to provide a check on quantitative estimates, and to ensure the isolation of any species failing to develop at the lower dilution. A medium of the following composition was employed: dextrose, 10 g.; peptone, 5 g.; KH_2PO_4, 1 g.; $MgSO_4.7H_2O$, 0·5 g.; agar, 20 g.; tap water, 1 l.; rose bengal, 1:15,000; reaction pH 6·8. Each soil horizon was sampled by a $5\frac{1}{2}$ in. length of $\frac{5}{8}$ in. diameter glass tubing, previously plugged with cotton-wool at both ends, wrapped in paper and sterilized at 160° C., for 1 hr. Each tube was used to sample six to eight different sections in the exposed horizon and was thereafter replugged; the contents were later transferred as a composite sample to a sterile, dry flask and thoroughly mixed by hand over hand shaking for 5 min. A 10 g. subsample was suspended in 490 ml. sterile distilled water to give an initial dilution of 1/50. This primary suspension was shaken end-over-end in a rotary shaker (32 rev./min.) for 5 min. The shaken suspension was allowed to stand for 30 sec. before 1 ml. portions were transferred to 97 ml. blanks of sterile distilled water to give an approximate dilution of 1/4900. This was shaken for 1 min. by hand and two 1 ml. portions removed. One was placed in a sterile Petri dish, the other was added to a dilution blank of 18·5 ml. to give a final dilution of 1/95,550. This final dilution aliquot was shaken by hand for 1 min. and a 1 ml. sample transferred to a sterile Petri dish.

Previously melted and cooled agar medium (15–20 ml.) was poured into each Petri dish and the contents thoroughly mixed by gentle rotation of the dish on the bench. Inoculated plates were incubated at room temperature (18–20° C.) for 30 days. Numbers of colonies developing were counted after 5 days. Thereafter isolation to pure culture was made and specific identification attempted. Eight replicate dilution plates were prepared from both 1/4900 and 1/95,550 dilutions of soil from each of three horizons of a brown forest soil under oakwood and from each of four horizons of a podzol soil under *Calluna* heath. Five screened immersion plates were buried in each of three horizons in the brown forest soil and in each of four horizons of the podzol soil during July 1950. The results are presented in Table 4.

Viewed in the light of general opinion, the comparison between these two isolation methods presented in Table 4 suggests that the dilution plate technique favours the isolation of species sporulating in soil, because in the

mechanical separation of soil crumbs by shaking, spores are more readily washed from such aggregates and brought into suspension than are hyphae closely investing soil components. Further, spore masses, particularly if formed of slimy spores, are readily separated into numerous units, each capable of developing into a colony on the culture plate. Mycelium, on the other hand, by the nature of its filamentous and possibly interwoven

Table 4. *A comparison of fungi isolated by screened immersion plates and by the dilution plate method*

	Screened immersion plates							Dilution plate method						
	Brown forest			Podzol				Brown forest			Podzol			
Fungus	A	B	C	A_2	B_1	B_2	C	A	B	C	A_2	B_1	B_2	C
Mucor hiemalis Wehmer	*	—	—	—	—	—	—	—	—	—	—	—	—	—
M. ramannianus Moeller	—	—	—	*	*	*	*	—	—	—	*	*	*	—
Zygorhynchus moelleri Vuill.	*	*	—	*	*	*	*	—	—	—	—	—	—	—
Mortierella alpina Peyr.	—	—	—	—	—	*	—	—	—	—	—	—	—	—
M. elongata Linm.	—	—	—	*	*	*	*	—	—	—	—	—	—	—
M. gracilis Linm.	*	—	—	*	*	*	*	—	—	—	*	*	—	—
M. humilis Linm.	*	*	—	—	—	—	—	*	*	—	—	—	—	—
M. marburgensis Linm	*	—	—	—	—	—	—	—	—	—	—	—	—	—
M. minutissima Linm.	*	—	—	—	—	—	—	—	—	—	—	*	—	—
M. nana Linm.	*	*	—	—	*	—	—	—	—	—	*	—	—	—
M. polycephala Coem.	—	—	—	*	*	—	—	—	—	—	—	—	—	—
Thielavia sp.	—	—	—	—	—	—	—	*	—	—	—	—	—	—
Rhodotorula sp.	—	—	—	—	—	—	—	—	*	—	—	—	—	—
Yeasts	—	—	—	—	—	—	—	*	—	—	—	—	—	—
Geomyces cretaceus Traaen	—	—	—	—	—	—	—	*	—	—	*	—	—	—
Cephalosporium acremonium Corda	—	—	—	—	—	*	—	—	—	—	*	—	—	—
Trichoderma viride Fr.	*	*	*	*	*	*	*	—	—	—	*	*	—	—
Gliocladium roseum (Link) Bainier	—	—	—	—	—	*	—	—	—	—	—	—	—	—
Aspergillus flavus Link.	—	—	—	—	—	—	—	—	—	—	*	—	—	—
A. repens (Corda) de Bary	—	—	—	—	—	—	—	—	*	—	—	—	—	—
A. versicolor (Vuill.) Tirab.	—	—	—	—	—	—	—	*	—	—	—	—	—	—
Penicillium frequentans Westl.	*	—	—	*	*	*	*	—	*	—	*	*	—	—
P. implicatum Biourge	—	—	—	*	—	—	—	—	—	—	—	—	—	—
P. melinii Thom	—	*	—	—	—	—	—	*	—	—	*	*	—	—
P. nigricans (Bain.) Thom	*	—	—	*	—	—	—	—	—	—	*	*	—	—
P. notatum Westl.	—	—	—	—	—	—	—	—	—	—	*	—	—	*
P. piscarium Westl.	*	—	—	—	—	—	—	—	—	—	—	—	—	—
P. pulvillorum Turfitt	—	—	—	—	—	—	—	—	—	—	—	—	*	—
P. roseo-purpureum Dierx	—	—	—	—	—	—	—	*	—	—	—	—	—	—
P. rubrum Stoll.	—	—	—	—	—	—	*	—	—	—	—	—	—	—
P. spinulosum Thom	—	*	—	*	—	—	*	—	—	—	—	—	—	—
P. thomii Maire	—	—	—	*	—	—	*	—	—	—	—	—	—	—
Penicillium sp.	—	—	—	—	—	—	—	—	—	—	—	*	—	—
Scopulariopsis (*sacculum* Dale)	—	—	—	—	—	—	—	*	—	—	—	—	—	—
S. diversispora van Beyma	—	—	—	—	—	—	—	*	—	—	—	—	—	—
Scopulariopsis sp.	—	—	—	—	—	—	—	—	—	—	*	—	*	—
Sporotrichum sp.	—	—	—	—	—	—	—	*	—	—	*	—	—	—
Acrostalagmus albus Preuss	—	—	—	—	—	*	—	—	—	—	—	—	—	—
Verticillium sp.	—	—	—	—	*	*	—	—	—	—	—	—	—	—
Botrytis cinerea Fr.	—	—	—	—	—	—	—	*	—	—	—	—	*	—
Hormodendron cladosporioides (Fres.) Sacc.	—	—	—	—	—	—	—	*	*	*	*	*	*	—
Oidiodendron fuscum Robak	—	—	—	—	—	—	—	*	—	—	—	—	—	—
Humicola grisea Traaen	—	—	—	*	—	—	—	—	—	—	—	—	—	—
Cylindrocarpon didymum (Hartung) Wollenw.	*	—	—	*	—	*	—	*	*	—	—	—	—	—
Sterile white mycelium	—	—	—	—	—	—	—	—	*	*	*	*	*	*
Sterile brown mycelium	—	—	—	—	—	—	—	—	—	—	*	—	—	—

hyphae, is more resistant to fragmentation and therefore is recorded with less frequency when numerical estimates are made from dilution plates.

The use of rich nutrient media in the dilution method must exercise a greater selective effect than that inherent in the screened immersion plate, employing plain water agar. In particular the type of carbohydrate employed has a strongly selective effect (Chesters, 1948; Garrett, 1951). Of those fungi capable of development on a given medium, the faster growing species are liable to overgrow the slower, preventing their isolation. More particularly on enriched media, strains of certain species may produce metabolic by-products, which may inhibit or encourage the growth of other species. This interplay in culture plates may lead to erroneous conclusions regarding the results from dilution plate studies. The concentration of the carbohydrate source (dextrose) and the temperature of incubation were found by Cohen (1950) to influence the development of fungi on isolation plates.

The dilution plate method provides little information regarding the micro-habitats of soil micro-organisms, because the soil structure is destroyed. Colonies which arise have developed from spores or from mycelium washed from inorganic and organic particles, and their distribution on plates bears little resemblance to the natural space relationships of these organisms in soil.

(5) *Screened immersion plate: soil plate* (Warcup)

In the dilution plate method soil particles generally do not contribute to the final results because they are usually discarded following further dilution of the primary suspension. Warcup (1950) plated-out discarded soil particles and obtained species of fungi which did not appear on plates prepared from the final dilutions of soil suspensions. He considered that these additional species had arisen from spores and hyphae which had not been dislodged from soil particles by the mechanical shaking of the original suspension. Cohen (1950) also recorded loss of species following further dilution of an initial suspension. In order to reduce such loss, Warcup proposed a plating method in which crushed soil aggregates or elementary suspensions of soil in sterile water were incorporated with an enriched agar medium in Petri dishes. An investigation comparing Warcup's soil plates with the screened plate technique was conducted in the following manner.

Random samples of soil were taken with sterile $4 \times \frac{1}{4}$ in. glass tubes thrust into freshly exposed soil layers. Each tube was replugged and brought to the laboratory where one end was opened, flamed and the first $\frac{1}{8}$ in. of soil removed and discarded. A small sample of soil, 0·1–0·2 g., was then removed with a flattened platinum needle to a sterile Petri dish, in which it was crushed and distributed evenly over the base of the dish. Successive $\frac{1}{8}$ in. layers of soil were discarded from tubes, and samples were taken as above in order to provide replicates; 10–15 ml. of previously melted and cooled sterile Czapek plus yeast extract agar were poured over the particles, and each culture dish was rotated gently on the bench to distribute the soil. After solidifying, the plates were inverted and incubated for 4 days at 24·5° C., when isolation of growth to pure culture was effected.

Thereafter plates were kept at room temperature (18–20° C.) for observation and isolation of additional fungi. The agar medium was prepared in two series, one having a reaction of pH 4·8 and the other of pH 6·8.

Twenty screened immersion plates were used in the 1–2 in. depth layer and ten screened plates in the 6–8 in. depth layer of the A horizon of a brown forest soil under species of *Pinus*. Three Warcup soil plates from each of the two media reaction series were inoculated for each of ten tubes

Table 5. *A comparison of fungi isolated by screened immersion plates and by the Warcup soil-plate method*

Species	Screened immersion plates	Soil plates
Mucor hiemalis Wehmer	*	—
M. Jansenni Lendn.	—	*
M. ramannianus Moeller	*	—
Zygorhynchus moelleri Vuill.	*	*
Absidia glauca Hagem	—	*
Mortierella alpina Peyr.	*	—
M. elongata Linm.	*	—
M. gracilis Linm.	*	—
M. humilis Linm.	*	*
M. jenkini (Smith) Naumov	*	—
M. minutissima Linm.	*	
M. vinacea Dix.–Stew.	*	*
Melanospora sp. (near *M. ornata* Zukal)	—	
Gymnoascus umbrinus Boud.	—	*
G. reesii Baranetsky	—	*
Pseudogymnoascus roseus Raillo	*	*
Yeasts	*	—
Phoma sp.	*	—
Pestalotia sp.	—	*
Cephalosporium acremonium Corda	*	*
C. asperum Marchal	—	*
Trichoderma viride Fr.	*	*
T. albus Preuss.	*	—
Paecilomyces sp.	*	—
Penicillium citrinum Thom	*	—
P. corylophilum Dierx.	*	—
P. cyaneo-fulvum Biourge	*	—
P. fellutanum Biourge	—	*
P. frequentans Westl.	*	*
P. janthinellum Biourge	*	*
P. lilacinum Thom	—	*
P. melinii Thom	*	*
P. nigricans (Bain.) Thom	*	*
Penicillium sp. (Herquei series)	—	*
Scopulariopsis (*sacculum* Dale)	—	*
Scopulariopsis sp.	—	*
Acremonium vitis Cattaneo	*	—
Sporotrichum sp.	*	—
Botrytis cinerea Fr.	*	—
Cylindrocarpon didymum (Hartung) Wollenw.	*	—
C. radicicola Wollenw.	*	—
Humicola grisea Traaen	*	*
Hormodendron cladosporioides (Fres.) Sacc.	—	*
Dicoccum asperum Corda	—	*
Cladosporium herbarum (Pers.) Link	*	—
Trichocladium opacum (Corda) Hughes	*	—
Oidiodendron fuscum Robak	*	—
? *Torula* sp.	*	—
Sterile mycelium	*	—

employed for sampling the 1–2 in. layer and for each of ten tubes sampling the 6–8 in. layer of the A horizon of the same soil, i.e. a total of 120 plates.

Soil conditions. Temperature, 7·5° C.; moisture (dry-weight basis), 8·8 %; reaction, pH 4·8.

Table 5 gives the species of fungi isolated by soil plate and screened plate methods in May 1950. Table 6 provides quantitative data concerning the species obtained by the two methods. Table 7 presents the fungi isolated by Warcup's soil plates employing media differing in pH.

Table 6. *Quantitative data concerning the species of fungi isolated by screened plates and by soil plates*

Screened plates	No. of isolations (from 30 plates)	Soil plates	Occurrence in 20 samples*
Sterile mycelium	24	*Mortierella vinacea*	20
Mortierella vinacea	23	*Penicillium nigricans*	20
M. humilis	17	*P. melinii*	20
M. elongata	13	*P. frequentans*	20
Humicola grisea	12	*P. janthinellum*	20
Cylindrocarpon didymum	12	*Trichoderma viride*	4
Penicillium melinii	7	*Zygorhynchus moelleri*	4
Mortierella gracilis	6	*Mortierella minutissima*	2
Trichoderma viride	6	*Cephalosporium acremonium*	2
Pseudogymnoascus roseus	4	*Mortierella humilis*	2
Mortierella minutissima	3	*Penicillium lilacinum*	2
M. jenkini	3	*Mucor jansenni*	2
? *Torula* sp.	3	Others	1 each
Others	1 each		

* A fungus was given a positive record if it occurred on one or more of the three plates prepared for each of 20 samples.

Table 7. *Fungi isolated from Warcup soil plates on Czapek medium at* pH 4·8 *and* pH 6·8

Isolated from both series	Isolated at pH 4·8	Isolated at pH 6·8
Zygorhynchus moelleri	*Mucor jansenni*	*Absidia glauca*
Mortierella vinacea	*Mortierella nana*	*Mortierella humilis*
Trichoderma viride	*Melanospora* sp.	*M. minutissima*
Penicillium janthinellum	*Gymnoascus umbrinus*	*Penicillium fellutanum*
P. lilacinum	*G. reesii*	*P. frequentans*
P. melinii	*Pseudogymnoascus roseus*	*Scopulariopsis* sp.
P. nigricans	*Pestalotia* sp.	*Dicoccum asperum*
	Cephalosporium acremonium	
	C. asperum	
	Humicola grisea	
	Hormodendron cladosporioides	

Table 5 shows that, of a total of forty-nine isolated species, twelve fungi were commonly isolated by both techniques, fifteen additional species were obtained on soil plates and a further twenty-one species were extracted with screened immersion plates. Table 6 shows a high frequency of *Penicillium* colonies on soil plates, a result only partially confirmed by screened plates.

Warcup (1951) employed an acid medium to isolate fungi from acid, near neutral and alkaline soils. Many fungi are sensitive to the reaction of the isolation medium, and so the use of an acid medium alone may have

prevented the isolation of some species. In the present study Czapek agar was prepared in two reaction series, pH 4·8 and pH 6·8; in addition to the several species isolated by both media, additional species were isolated in the one series and not in the other (Table 7).

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CILIOCHORELLA SYDOW, *PLAGIONEMA* SUBRAM. & RAMAKR., AND *SHANORIA* GEN.NOV.

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(With 4 Text-figures)

A study has been made of the genus *Ciliochorella* Sydow, represented by the two species, *C. mangiferae* Sydow (Type) and *C. bambusarum* Shanor. Examination of the type specimen of the former showed that the conidia are phragmospores with one apical, one basal and one lateral appendage and not amerospores with two apical and a persistent tail-like conidiophore as described by Sydow. The generic description of *Ciliochorella* is accordingly emended. It is also shown that *C. mangiferae*, as re-described here, is an earlier valid name for *Plagionema indica* Subram. & Ramakr. *Ciliochorella bambusarum* is shown to be not congeneric with *C. mangiferae* and is made the type of a new genus of the Sphaeropsidales, *Shanoria*.

The genus *Ciliochorella* was described by Sydow, with *C. mangiferae* as the type species (Sydow & Mitter, 1935, p. 62). The fungus was collected on dead leaves of *Mangifera indica* L. at Allahabad, India. Sydow's description of the genus was as follows:

Stromata typicae phyllachoroidea, orbiculari-lenticularia vel biconvexa, utrinque clypeo opaque atro-brunnea, prosenchymatice contexto omnino clauso in maturitate irregulariter rupta praedita. Conidiophora in parte basali tantem evoluta, ad basim conidiorum ut appendiculam ciliiformem adherescentia. Conidia copiosissima, cylindracea, subhyalina, in cumulo tantem dilutissimae flavo-brunnea, continua, ad apicem ciliis duobus filiformibus valde divergentibus praedita.

The conidia of *C. mangiferae* were described by Sydow as being one-celled and hyaline, and as having two divergent apical appendages and a persistent tail-like conidiophore.

The type material of this fungus was not available for our study from the Herbarium of the U.S. Department of Agriculture at Beltsville, from Sydow's collections at the Naturhistoriska Riksmuseet, Stockholm, or from the Herbarium Cryptogamae Indiae Orientalis at New Delhi. However, a small fragment of the type material was available at the Department of Botany, University of Allahabad, where part of J. H. Mitter's collections have been deposited. Through the courtesy of Dr R. K. Saksena, Reader in Botany, University of Allahabad, a slide from the type was made available to us. The material had an abundance of conidia. Our study of this material showed that the fungus was not correctly described by Sydow. The conidia are 3-septate and not 1-celled as he described (Fig. 1). The conidiophore does not persist attached to the base of the conidium as a tail-like appendage. On the other hand, the basal cell of the conidium tapers into a hyaline, slender, pointed, straight or curved appendage. The conidio-

phores are hyaline and lageniform, each with a stout, short base and a slender neck (Fig. 2) and do not persist attached to the conidia, which are deciduous. In fact, the type material on which Sydow's description was based agrees, in every way, with that of *Plagionema indica* Subram. & Ramakr., recently described by us as the type of a new genus (Subramanian & Ramakrishnan, 1953). It is, therefore, necessary to emend the description of *Ciliochorella* Sydow, which now appears to be an earlier name for *Plagionema* Subram. & Ramakr.

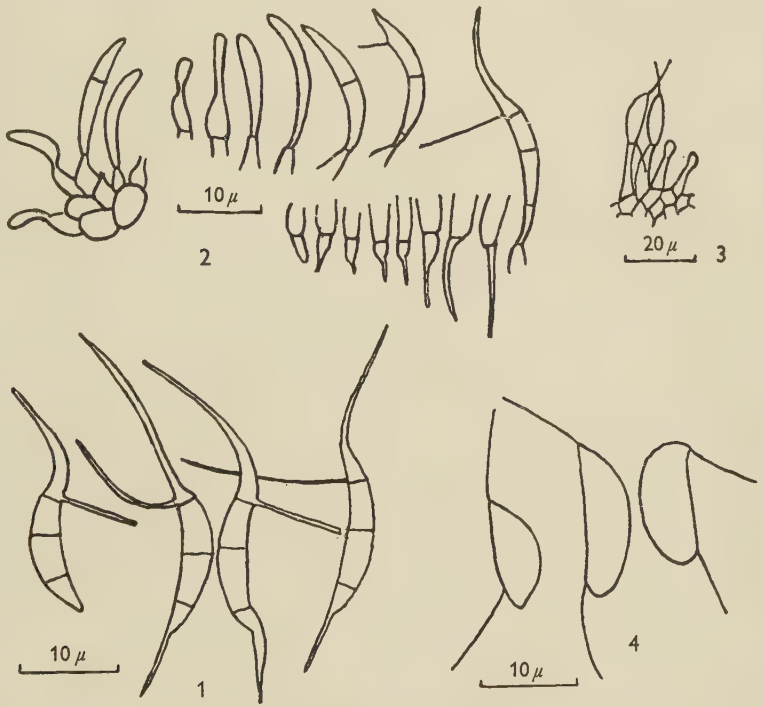

Fig. 1. Conidia of *Ciliochorella mangiferae* (from the type collection, Herb. M.U.B.L. no. 1356).

Fig. 2. Conidiophores and developmental stages of conidia of *Ciliochorella mangiferae* (redrawn from Subramanian & Ramakrishnan, 1953).

Fig. 3. Conidia, conidiophores and associated cells of *Shanoria bambusarum* (redrawn from Shanor (1946), fig. 8*b*).

Fig. 4. Conidia of *Shanoria bambusarum* (from the type material, Herb. M.U.B.L. no. 1269).

Ciliochorella Sydow char.emend.

Synonym: *Plagionema* Subram. & Ramakr., 1953, in *J. Indian bot. Soc.* **32**, 135, *ic.*

Pycnidia nigra, circularia, dimidiata, dispersa, intra-epidermalia, astoma, ex centro per fissuras radiantes aperientia, circa marginem haud dehiscentia, unilocularia, pariete pseudo-parenchymatico circumdata, dimidium superius ejus sterile, inferius fertile. Conidiophora erecta, ex dimidio inferiore surgentia. Conidia hyalina, dorsiventralia, paullum curvata, 4-cellulata, cellula apicalis cujusque in caudam longam filiformen producta, et prope basim ejus in latere concavo, appendice laterali, longa, hyalina ornata; cellula basalis cujusque appendice terminali, hyalina, tenui, acuta, recta vel curvata praedita.

Type species: *C. mangiferae* Sydow apud Sydow & Mitter, 1935, *Ann. mycol., Berl.*, **33**, 63

Synonym: *Plagionema indica* Subram. & Ramakr., 1953, in *J. Indian bot. Soc.* **32**, 136, *ic.*

An emended description of the fungus is:

Pycnidia amphigenous, black, circular, with a slightly raised central point, scattered, dimidiate, intra-epidermal, astomous, opening by cracks radiating from the centre, unilocular, with a pseudo-parenchymatous wall all round, the upper half sterile, the lower fertile. Conidiophores arising from the lower half of the wall, erect, lageniform, with a short, stout base and a slender neck, $7 \times 3{\cdot}5$ μ; conidia hyaline, dorsiventral, slightly curved, 4-celled, cylindrical in the middle, and narrowed towards either end, apical cell of the conidium tapered into a long, hyaline, usually curved, filiform tail and bearing, on its concave side, near its base a long, hyaline lateral appendage measuring 20 (16–24) μ; the basal cell of the conidium tapering into a hyaline, slender, pointed, straight or curved appendage measuring 7 (1–10) μ; conidia, 39×3 ($31–45 \times 2{\cdot}6–3{\cdot}3$) μ.

A more detailed description of this fungus, including details of the development of the conidia and their appendages, has been given by Subramanian & Ramakrishnan (1953, figs. 1–23).

Type specimen. On dead leaves of *Mangifera indica* L., Allahabad, India, no. 98. Slide in Herb. M.U.B.L. no. 1356, ex Herbarium, Botany Department, University of Allahabad.

Other collections: In addition to the twelve collections (Herb. M.U.B.L. nos. 354–356, 519–521, 553, 557–560 and 612) listed by Subramanian & Ramakrishnan (1953, pp. 134–5), the following additional collections have been made by us: on dead leaves of *Couroupita guianensis* Aubl. (Lecythidaceae) [M.U.B.L. no. 645], of *Terminalia* sp. (Combretaceae) [no. 646], of *Swietenia* sp. (Meliaceae) [no. 647], Government Museum campus, Egmore, Madras, 9 December 1951, Coll. C.V.S.; on dead leaves of *Glycosmis cochinchinensis* Pierre (Rutaceae), Christian College campus, Tambaram, Madras State, 6 January 1952, coll. K.R. & C.V.S., no. 683; dead leaves of *Azadirachta indica* A. Juss. (Meliaceae) [no. 1344], of *Semecarpus anacardium* Linn. f. (Anacardiaceae) [no. 1345], Narsapur, Hyderabad-Deccan, 22 August 1955, coll. C.V.S.; on dead leaves of Rubiaceous plant, Narsapur, Hyderabad-Deccan, 22 August 1955, coll. C.V.S. (no. 1346); on dead leaves, University Botany Laboratory garden, Madras, 7 November 1951, coll. C.V.S. [no. 620], Government Museum campus, Egmore, Madras, 9 December 1951, coll. C.V.S. [no. 644], Christian College campus, Tambaram, Madras State, 6 January 1952, coll. K.R. & C.V.S. [no. 686], Marina, Chepauk, Madras, 30 July 1955, coll. K.R. [no. 1285]; on dead pods of legume (Leguminosae), Narsapur, Hyderabad-Deccan, 22 August 1955, coll. C.V.S. [no. 1347].

The only other fungus assigned to *Ciliochorella* so far is *C. bambusarum* Shanor (Shanor, 1946), on leaves of *Arthrostylidium racemiflorum* Steud. collected from El Salvador. We have carefully studied the original description, and the figures of this species, and have also examined, through the courtesy of Dr J. A. Stevenson of the United States Department of

Agriculture and of Dr Leland Shanor of the University of Illinois, the type specimen. In this fungus the pycnidia occur as locules in an elongate stroma which develops within the leaf tissue, usually nearer one or the other of the epidermal layers. The conidia are produced acrogenously on cylindrical conidiophores lining the cavity of the pycnidium (Fig. 3). The conidia are hyaline, one-celled, clavate to navicular, and have one straight or slightly curved subapical cilia-like appendage at each end of the conidium (Figs. 3, 4).

It will be obvious from the above description that *C. bambusarum* is not congeneric with *C. mangiferae* as re-described by us in this paper, or even as erroneously described by Sydow (Sydow & Mitter, 1935); for the conidia in *C. bambusarum* have only one appendage, each at the base and at the tip, a lateral appendage being absent. Further, in contrast to *C. mangiferae*, the conidia are not four-celled but one-celled. *C. bambusarum* is, therefore, not a *Ciliochorella*.

We find that there is no genus of the Sphaeropsidales which can accommodate *C. bambusarum* and, therefore, propose a new genus for it, named after Dr Leland Shanor who first described it.

Shanoria Subram. & Ramakr. gen.nov.

Stromata nigra, carbonacea, uni- vel pluri-locularia, conidiophores vestiti, in maturitate rima irregulari longitudinali dehiscentes. Conidiophora cylindrica vel clavata. Conidia hyalina, continua, utrinque sub apice setula prominenti recta vel curvata praedita. Fungus imperfectus, Sphaeropsidales, Hyalosporae.

Stromata black, carbonaceous, with one or more loculi, lined with conidiophores, at maturity dehiscing by irregular longitudinal rupture. Conidiophores cylindrical or clavate. Conidia hyaline, continuous, with one subapical appendage at each end.

Type species: **Shanoria bambusarum** (Shanor) Subram. & Ramakr. comb.nov.

Basonym: *Ciliochorella bambusarum* Shanor, 1946, in *Mycologia*, **38**, 335, figs. 6, 8.

Stromata black, single or aggregated, oval to linear, subconvex, carbonaceous, innate, up to 2·5 mm. long and 0·5 mm. broad, outer wall carbonaceous and prosenchymatous, composed of angular to subglobose cells: inner wall thinner and non-carbonaceous; loculi 1–4; invested with conidiophores, dehiscing irregularly and longitudinally at maturity; conidiophores cylindrical to narrowly clavate, 10–26 × 2–2·5 μ; conidia abundant, narrowly clavate to navicular, hyaline, one-celled, truncate, 14–16·5 × 4·7–5·2 μ, with a prominent subapical, straight or curved appendage at each end; appendages 9–15 μ long.

Type collection. On leaves of *Arthrostylidium racemiflorum* Steud. (Gramineae), John Tucker No. 750, coll. 8 January 1942, south side of Cocoquatique, El Salvador, ex U.S.D.A. Mycological Collections (Herb. M.U.B.L. no. 1269—slide).

We are deeply indebted to Dr R. K. Saksena, Department of Botany, University of Allahabad, for a slide of the type material of *Ciliochorella mangiferae*, and to Dr J. A. Stevenson of the U.S. Department of Agriculture, Beltsville, and Dr Leland Shanor of the University of Illinois, for fragments from the type collection of *Ciliochorella bambusarum*.

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ADDENDUM

Since this paper went to press, B. L. Chona & R. L. Munjal (*Indian Phytopath.* **8**, 74–75, 1955) have reported that *Plagionema* is a synonym of *Ciliochorella*. They have stated that *Plagionema* agrees largely with Sydow's description of *Ciliochorella* and that the conidia of *Ciliochorella* are two-celled with the central septum rather inconspicuous. It is quite obvious that Chona & Munjal's understanding of Sydow's description of *Ciliochorella* and their interpretation of the spore-septation in the genus are both erroneous and misleading. Indeed, we have nothing to add to or alter in our original text.

Trans. Brit. mycol. Soc. **39** (3), 319–329 (1956).

TOMATO SEED TRANSMISSION OF *DIDYMELLA LYCOPERSICI* KLEB.

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(With 2 Text-figures)

An account is given of the symptoms of *Didymella* stem rot of tomatoes as it affects the outdoor crop in Jersey. The results of tests and field experiments with infected or contaminated seed suggest that this is probably rarely important as a source of stem rot. Pycnospores of *D. lycopersici* did not survive on the surface of artificially infected seeds for more than about 9 months. Similarly, the fungus appeared to die out within a year of extraction on affected commercial seed. Tests on new season's commercial seed revealed the presence of *D. lycopersici* on almost 30% of the samples examined, whereas the fungus was not found on any samples that were tested more than a year after extraction. Field experiments in which plants were raised from both lightly and heavily infected samples of tomato seeds failed to establish any connexion between the presence of *D. lycopersici* on the seed and stem rot within the crop.

Stem rot of tomatoes, caused by the fungus *Didymella lycopersici* Kleb., is the most destructive of all diseases of the outdoor tomato crop in Jersey, where it was first officially recorded (as tomato canker) in 1926 (Simpson, 1927).

Symptoms

D. lycopersici occasionally causes a foot rot of young plants in the propagating stages; indeed, in Jersey, cases of this kind are observed every year and are probably commoner than is usually suspected. The stem bases of the affected plants become dark brown and rotten at and below the ground, and pycnidia of the fungus may often be seen in large numbers on the brown lesions.

After planting out, two types of attack occur. In the earlier, primary attacks, usually attributed mainly to infection from the soil (Orth, 1939), or the seed (Hickman, 1946), the base of the stem, and sometimes also the hypocotyl and the upper part of the main root (but rarely the lateral roots) shows a slightly shrunken, dark brown scar, which eventually girdles the stem, and may be 2 in. or more in length. The epidermis and cortex of the stem become soft and rotten, and pycnidia are frequently visible. Browning of the vascular tissues and of the pith may develop for several inches above and below the region of the scar. Later, the lower leaves may yellow and the plant suddenly wilts and dies. Indeed, this sudden wilting is often the first indication of the presence of the disease, although some time before they collapse, the diseased plants may become markedly purplish in colour. Recently in Jersey, among plants raised in

artificially infested soil, wilted plants have occasionally been found on which no external lesion was visible, but in which vascular browning was observed towards the base of the stem. *D. lycopersici* was isolated from the brown vascular tissues of these plants. Primary attacks on young plants sometimes cause death within 2–4 weeks of transplanting in the open, but sometimes the affected plants produce fresh roots above the diseased lesion, and the new roots enable them to grow apparently normally for several weeks before they wilt and die. The fact that the plants were affected when young may then be obscured.

Later in the season, further lesions (normally regarded as secondary) may appear on almost all the aerial parts of the plant. Large dark brown scars like those already described arise on the main stems above soil level, often where side-shoots have been removed. The petioles of the leaves are often attacked, and the fungus may grow back from the leaf into the stem, but the lamina of the leaf is very rarely infected. In secondary attacks, destruction of the fruit may also take place. Although infection may occur on any part of the fruit, it is most frequent under the calyx, and the proximal end of the fruit then becomes covered by a black crust containing many pycnidia. The fungus also spreads within the interior of the fruit, blackening its tissues, and sometimes infecting the seeds (Orth, 1939; Fisher, 1954). Diseased fruits usually fall from the plant.

Sources of the disease

Possible sources of *Didymella* stem rot include internally infected or externally contaminated seed, propagating soil, or soil in the field or glasshouse borders, propagating boxes and pots, supporting canes and stakes, glasshouse structures, contaminated water supplies, spores spread from plant to plant within a crop by rain or by watering, air-borne spores arising from diseased crops or from heaps of old diseased tomato haulms, and spores carried from plant to plant by insects and other small animals and on the hands and tools of workers within the crop.

The relative importance of these many possible sources of disease is unknown, and some remain quite unexamined. In a general investigation of glasshouse water supplies, Bewley & Buddin (1921) isolated a number of common phytopathogenic fungi, but *D. lycopersici* was not among them. Since the pycnospores of the fungus are of the sticky type usually regarded as primarily splash-borne or carried by insects (Mason, 1937; Langeron, 1945), it seems reasonable to suggest that stem rot may be spread from plant to plant within a crop by rain or watering, and also by insects and other small animals. These aspects of the matter have received little consideration, though Small (1943) has shown that stem rot may enter a crop through the dipping of boxes of young plants in water carrying spores of *D. lycopersici*, before planting out. When G. E. Thomas and the present writer took spring-tails that had been feeding on tomato plants affected by stem rot, and allowed them to walk on agar plates, colonies of *D. lycopersici* developed on the agar. Many authors (R., 1925; Kordes, 1933; Bremer, 1933; Small, 1940) have pointed to the use of old, contaminated supporting canes as a possible source of stem-rot attacks, and

Hickman (1946) was able to show that the disease could indeed be carried in this way.

Recent investigations on *Didymella* stem rot in Jersey have included studies on seed transmission of *D. lycopersici* and its effects, the persistence of the fungus in field soils, and its spread through the air, with particular reference to heaps of old tomato refuse. The present paper deals with the results of the work on seed transmission.

Seed transmission: introduction

Evidence concerning the view that *Didymella* stem rot of tomatoes may be seed-transmitted has been conflicting and difficult to interpret. Schoevers (1929) published the results of experiments with seed derived from a batch from which a commercial grower had previously raised diseased plants. Schoevers treated some of the seed with Uspulun dust (a mercurial preparation), and then placed the treated and untreated seed, some in Petri dishes on moist filter-paper, and some in clean, moist sand. In the Petri dishes, pycnidia of *D. lycopersici* were produced on the untreated seeds, but not on the treated ones, while in the sand, the untreated seeds gave rise to diseased seedlings, whereas the seedlings from the treated seeds were healthy. After storage in the laboratory for about 4 months, seeds from the same sample were sown and plants raised from planting outdoors, but no plants were attacked by *D. lycopersici*, and Schoevers concluded that the fungus on the seed had been unable to withstand the dry, cold conditions of storage.

Small (1937) isolated the fungus from seed obtained from infected fruits, but when he raised plants from seeds freshly extracted from diseased fruits, and from seeds artificially inoculated with pycnospores, the plants were all healthy. Sheard (1944) also raised plants from infected seed, and although she kept some of these plants in pots for 3 months, none showed signs of disease. Similarly, Orth (1939) states that the testa of the tomato seed may be permeated by mycelium and covered with pycnidia, but the embryo is rarely attacked, and infected seed rarely gives rise to diseased plants.

When Small (1939) carried out further experiments, however, in which once more he raised seedlings from naturally and artificially infected seeds, he found that though in all cases nearly all the seedlings were healthy, an occasional seedling was diseased, and he pointed out that as such a plant gave rise to an enormous number of spores, it might spread disease to other plants raised in the same propagating box, and so cause stem rot in these plants at a later stage. A similar view was put forward by Ogilvie (1945), who found that infected seed germinated under very moist conditions gave rise to a small percentage of diseased seedlings, and that when the seedlings were grown on, many became secondarily infected.

Seed collected from commercial growers was tested for the presence of *D. lycopersici* by Small (1937), and by Hickman (1946). Small tested seed from four growers, but found the fungus only in one doubtful instance, whereas Hickman, who tested seed from ten growers, isolated the fungus from four of his ten samples.

Hickman also raised plants from heavily infected seed and from non-infected seed mixed with 2 % of the badly infected sample. Three months after planting out in the field, about one-third of the plants from the first and about a quarter of those from the second batch of seed had been attacked at the foot by *D. lycopersici*; Hickman therefore suggested that a small number of infected seeds in a sample might have a large effect. In this experiment, however, no plants were raised from non-infected seed alone, so that the disease possibly entered the crop from some source other than the seed.

Experimental

In an attempt to clarify some of the points raised above, the following investigations were carried out:

(*a*) Experiments on the longevity of pycnospores of *D. lycopersici* on the surface of artificially contaminated seeds.

(*b*) Tests on samples of commercial tomato seeds (as extracted by Jersey growers) for the presence of *D. lycopersici*.

(*c*) Experiments to assess the influence of contaminated or infected seed on the incidence of stem rot infection in the field.

(*a*) *The longevity of pycnospores on artificially contaminated seeds*

A number of experiments were carried out on the longevity of naked pycnospores of *D. lycopersici* on the surface of tomato seeds, which were contaminated by wetting with a heavy but unstandardized suspension of pycnospores. To obtain the spore suspension, a section of fresh tomato stem including a large stem rot lesion covered with pycnidia was washed vigorously under a strong jet of tap water, and then rinsed in three changes of sterile distilled water. The stem section was then placed in further sterile distilled water, into which oozed coils of pycnospores. Drops of water were examined from time to time, and when numerous spores could be observed in any drop taken, the suspension was decanted off. The tomato seeds to be contaminated were placed in a beaker, into which was poured enough spore suspension to cover them. The seeds were stirred and shaken until thoroughly wetted, placed on a glass sheet to dry, and then placed in envelopes and stored in the laboratory. From time to time they were tested for the presence of *D. lycopersici* by placing on Coons' agar, a synthetic medium containing: maltose, 7·0 g.; potassium nitrate, 2·0 g.; magnesium sulphate, 1·2 g.; potassium acid phosphate, 2·7 g.; agar, 15·0 g.; water, 1000·0 ml. *D. lycopersici* grows well and produces characteristic colonies in this medium.

The results of four experiments are summarized in Fig. 1. In the first and second experiments, in which fifteen and fifty seeds respectively were tested on each occasion, the seeds were placed on slopes in individual test-tubes by means of a platinum loop which was flamed and cooled before lifting each seed. In two further experiments (Exps. 3 and 4), samples each of 100 seeds were plated at intervals on to Coons' agar in 9 cm. Petri dishes, ten seeds per dish, the seeds being handled with forceps which were flamed and cooled before lifting each ten seeds.

It will be seen from Fig. 1 that with time there was a decline in the number of seeds from which *D. lycopersici* was recovered, and in no case was the fungus recovered after a period of more than 252 days (approximately 9 months).

(*b*) Didymella lycopersici *on commercial tomato seeds*

The tests on commercial tomato seed were all carried out by the agar-plate method (derived from the 'Ulster method' of Muskett & Malone, 1941), 100 seeds from each sample being plated on Coons' agar in 9 cm. Petri dishes. Ten seeds were placed in each dish, the seeds being handled with sterile forceps which were resterilized by dipping in industrial spirit, flaming and cooling before plating each ten seeds. The plates were kept at laboratory temperature for 4 weeks. Characteristic colonies developed

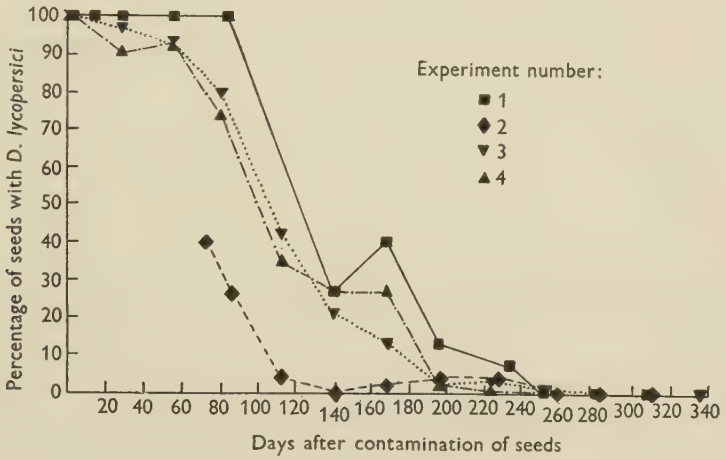

Fig. 1. Longevity of pycnospores of *Didymella lycopersici* on the surface of artificially contaminated tomato seeds.

on and in the agar around affected seeds, and pycnidia were produced after 1–3 weeks. Many unwanted moulds appeared upon the plates, and some of them, particularly a species of *Mucor*, in some tests overgrew other fungi. The destruction of these moulds by surface sterilization before plating could not be carried out, as it would have led to the removal of any adherent spores of *D. lycopersici* also. The seeds were therefore plated without prior surface sterilization. Resultant colonies that in any way resembled *D. lycopersici* were subcultured as soon as possible, and subcultures later appearing to be that fungus were inoculated into tomato plants to see if they produced symptoms of stem rot, and typical pycnidia and pycnospores, on the living plants. This inoculation procedure was also used for colonies over-run on the plates by other fungi. In this event, if *D. lycopersici* was present together with harmless saprophytes, the latter failed to grow on the living tomato plant, whereas the former gave rise to a characteristic stem rot lesion in about 1 week.

The seeds were all extracted by the growers by the fermentation method (Bewley, 1950), and each sample consisted of approximately 2000 seeds, which were stored in sealed envelopes in the laboratory until required. In view of the results obtained with artificially contaminated seeds, three series of tests were carried out. In the first, samples of seeds were examined for *D. lycopersici* within a year (and as far as possible within a few months) of extraction. These tests were carried out to find whether the fungus was commonly present in new season's tomato seed in Jersey. In a second series, samples were collected and examined more than a year after extraction to see if significantly fewer were contaminated with the fungus than in the first series. In a third, small series, commercial samples on which *D. lycopersici* had been found were retested at intervals until the fungus could no longer be found upon them.

Table 1. Didymella lycopersici *on commercial tomato seed*

	Samples tested within a year of extraction		Samples tested more than a year after extraction	
Year of extraction	No. tested	No. yielding *D. lycopersici*	No. tested	No. yielding *D. lycopersici*
1949	—	—	4	0
1950	13	2	10	0
1951	39	9	10	0
1952	59	20	7	0
1953	—	—	19	0
Totals	111	31	50	0

χ^2 tests. (1) All samples: $\chi^2 = 15{\cdot}544$ ($n=1$) (significant at the 0·1 % level). (2) Samples extracted in 1950, 1951 and 1952 only: $\chi^2 = 8{\cdot}188$ ($n=1$) (significant at the 1 % level).

For the first series of examinations, samples of seed were collected from growers in Jersey for three successive seasons. Thirteen samples were collected from the 1950 crop (extracted between September and November 1950). Collection in this case could not be made until March 1951, and the testing of the seed was carried out throughout April, May and June of the same year (i.e. from about 5–9 months after extraction). Similar tests were made on samples of newly extracted seed collected in the autumn of 1951 and 1952. Thirty-nine samples of 1951 seed and fifty-nine of 1952 seed were examined. These tests were carried out as soon as possible (in 75·5 % of the cases between 1 and 3 months, and in 99·8 % of the cases between 1 and 4 months) after extraction.

For the second series, fifty samples of older seed of various ages were collected and tested in November and December 1954.

The results of these two series of tests appear in Table 1. It will be seen that *D. lycopersici* was found to be present on about 28 % of the new season's seed, whereas the tests failed to show the presence of the fungus on any of the samples of older seed. The results of the two series were compared with one another by means of χ^2 tests, one of which dealt with the figures as a whole, while a second was restricted to the samples extracted in the years 1950, 1951 and 1952, since samples from those years only were common to the two sets of tests. The results of the χ^2 tests are incorporated in Table 1.

It may be noted here that the number of seeds per subsample from which *D. lycopersici* was isolated was usually small. Among the thirty-one samples of the first series shown to be affected, in twenty-four the fungus was isolated from between one and five of the 100 seeds plated, while in six cases, between six and ten seeds were affected, and in the remaining subsample, eighteen. These figures are too few in number to use as the basis for any final conclusions, but they do not suggest that infection of individual commercial seed samples with *D. lycopersici* is usually heavy.

The question of the longevity of *D. lycopersici* on commercial tomato seeds was examined in a different way by means of a further small series of tests, in which eight out of nine samples (the ninth being rejected owing to its small size) of seed extracted in 1951, and from which the fungus had been isolated, were retested at intervals. The tests were continued until the fungus failed to appear for at least two and usually three successive testings.

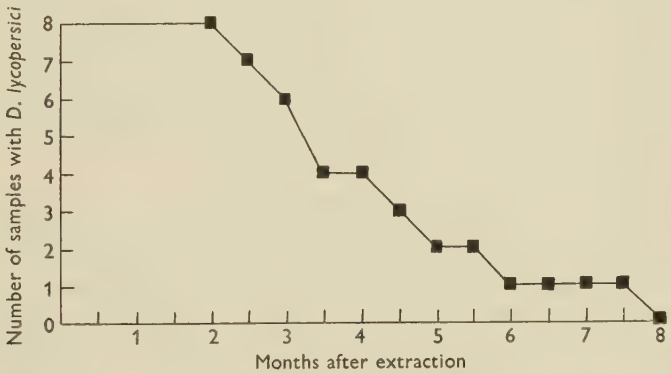

Fig. 2. Longevity of *Didymella lycopersici* on naturally contaminated or infected tomato seeds.

From the results, a graph (Fig. 2) was constructed, showing the number of samples known to be still infected or contaminated at the various given times. The times after extraction are approximate only, since few growers were able to supply the precise extraction dates of their samples.

Although the results of these tests are based on only eight samples, they supplement and help to confirm those reported above for artificially inoculated seeds, and those obtained by comparing newly extracted and old seed, since, with time, they show a steady decline in the number of infected samples, none of which was found after more than about $7\frac{1}{2}$ months after extraction.

(*c*) *Influence of contaminated or infected seed on the incidence of stem-rot infection in the field*

To examine the effect under field conditions of raising tomato plants from seed contaminated or infected by *D. lycopersici*, two field experiments were carried out. In these experiments, plants of the variety Sunrise were raised in partially sterilized soil and planted in randomized blocks in land

in which no tomatoes had been grown for at least 15 years. The plots within each block contained forty plants in two double rows 3 ft. apart, the two rows of each double row being 1 ft. from one another. The plants in the rows were 15 in. apart. The plants were raised from the seed samples detailed below, and each plant group was replicated five times. No diseased plants were found in the propagating stages in either experiment. After planting out, the plots were examined at frequent intervals for the presence of stem rot, and diseased plants were removed when seen, to reduce as much as possible the spread of disease from plant to plant.

The first experiment was planted-up on 26 May 1953, with plants raised from the following samples of seed:

Groups A and a. From seed tested on agar, and from which *D. lycopersici* was not isolated.

Groups B and b. From infected or contaminated commercial seed. Five months before sowing, a sample of the seed was tested on agar, and *D. lycopersici* was isolated from 5 of the 100 seeds examined.

Groups C and c. About 2 weeks before sowing, the seed for these groups was inoculated with pycnospores of *D. lycopersici* by dipping in a heavy spore suspension, and then drying on a glass plate before storing. On testing a sample of the inoculated seed on agar at sowing time, *D. lycopersici* was recovered from all of the 100 seeds tested.

At the time of sowing, therefore, one sample of seed was free of *D. lycopersici*, a second was at the most lightly infected or contaminated, while the third was heavily contaminated with pycnospores. The seed for groups a, b and c was dusted with a proprietary seed protectant containing 5% tetramethyl thiuram disulphide (thiram), the dust in the case of group c being applied after inoculation and drying of the seed.

The results of the experiment appear in Table 2. The figures given include both the numbers of plants with stem rot at or below ground-level, and the total number of plants with stem rot either at the foot or elsewhere, since it seems likely that the attacks at ground-level or below are largely primary, and due to infection from soil (Orth, 1939), or seed (Hickman, 1946), whereas the attacks elsewhere on the plant are secondary in origin. The number of diseased plants was small, even 15 weeks (105 days) after planting, and a statistical analysis of the results failed to demonstrate any significant differences between the groups of plants, either in the numbers of plants attacked at the foot only, or in the total numbers of affected plants. It is unlikely that the smallness of the losses experienced was due to climatic conditions, as among plants grown throughout approximately the same period on the same farm, on other plots in which the soil had been artificially inoculated with *D. lycopersici*, losses from stem rot were extremely heavy.

In the second experiment, planting took place on 21 May 1954, and the plants were raised from the following seed samples:

Group D. From seed inoculated with spores of *D. lycopersici* by dipping in a spore suspension, and then drying on a glass plate before storing. A sample of this seed was tested at the time of sowing, and *D. lycopersici* was isolated from 5 of the 100 seeds tested.

Group E. From commercial seed, from 1 % of a sample of which *D. lycopersici* was isolated approximately 5 months before sowing.

Group F. From seed a sample of which was tested, but from which *D. lycopersici* was not isolated.

Two further groups, G and H, were raised from seed similar to that of group F, and these plants were set out in plots of which the soil was inoculated with *D. lycopersici* by digging in diseased haulms before planting. Details relative to these groups will be given and discussed elsewhere (Phillips, 1956).

Table 2. *Influence of contaminated or infected seed on the incidence of stem rot infection in the field*

Time after planting (days)	No. plants with stem rot (means of 5 replicates of 40 plants)											
	With stem rot at foot.						With stem rot at foot or elsewhere					
	A	a	B	b	C	c	A	a	B	b	C	c
90	0·2	0·4	0·4	0	0·2	0·2	0·2	0·4	0·6	0·2	0·2	0·2
105	0·6	1·0	0·6	0·2	0·8	0·4	1·8	2·8	2·2	1·0	1·0	1·4

A, a: seed free of *D. lycopersici*; B, b: seed (commercial) with *c.* 5 % infection when tested; C, c: seed inoculated with *D. lycopersici* (100 % infection at sowing). A, B, C: untreated seed; a, b, c: seed dusted with thiram.

Table 3. *Influence of contaminated or infected seed on the incidence of stem rot infection in the field*

Time after planting (days)	No. plants with stem rot (means of 5 replicates of 40 plants)					
	With stem rot at the foot			With stem rot at foot or elsewhere		
	D	E	F	D	E	F
50	0	0	0	0	0	0
52	0·2	0	0	0·2	0	0
62	0·4	0	0	0·6	0	0
67	0·6	0	0	0·8	0·4	0·4
74	1·6	1·0	0·6	2·4	2·8	2·4
81	1·6	1·2	1·0	3·2	4·4	3·6
89	2·0	1·6	1·6	5·8	7·6	6·0
98	3·8	4·2	5·6	12·8	15·0	17·4
115	3·8	4·4	5·8	21·4	26·2	27·6

D, seed inoculated with *D. lycopersici* (5 % infection at sowing time); E, seed (commercial) with 1 % infection by *D. lycopersici* when tested 5 months before sowing; F, seed free of *D. lycopersici*.

The results of the section of this experiment dealing with infected seed are summarized in Table 3. In this experiment, losses were considerably higher than in the first, spread of disease from the plants growing in inoculated soil (in which losses were very heavy) taking place in the later stages of the experiment. Nevertheless, the numbers of plants with stem rot at or below ground-level remained low to the end of the experiment. A statistical analysis of the results was carried out, and as in the previous experiment, no significant differences could be found between the numbers of diseased plants (either at the foot or elsewhere) in either group raised from affected seed, and those in the control group grown from seed free of *D. lycopersici*.

Discussion

The results of the above tests on commercial tomato seed agree with previous work (Hickman, 1946) in demonstrating that *D. lycopersici* is a common fungus on such seed, though the percentage of affected seeds in a sample is probably in most cases small. The experiments carried out on the longevity of the pycnospores on artificially contaminated seed and on affected commercial samples, including comparison of the proportions of infected samples in new and old seed, indicate that with time there is a rapid fall in the proportion of infected seeds, and provide evidence that *D. lycopersici*, while frequently present on freshly extracted seed, is likely to be rare in samples over a year old. The position here is therefore comparable to that with *Septoria apii* Chester, the pycnospores of which on the seeds of celery do not survive beyond the second year (Campanile, 1926). In consequence, the use of two (or more)-year-old seed has often been advocated as a control measure for celery leaf spot, since the celery seed remains viable longer than the fungus spores. A similar suggestion has been made for the control of blind seed disease of rye grass (Noble, in Western, 1945), caused by *Phialea temulenta* Prill. & Delacr., in which also the seed remains viable longer than the parasite.

In the case of tomato stem rot, however, so far no satisfactory evidence has been found that seed samples infected or contaminated by *D. lycopersici* give rise to diseased crops. The literature contains no account of any controlled experiment in which affected seed significantly increased numbers of diseased plants in the field. Schoevers (1929) and Ogilvie (1945) were able to show that diseased seedlings could arise from affected seeds, but Schoevers states that his seedlings were perforce raised under cold, unsatisfactory growing conditions, while Ogilvie raised his seedlings under very moist conditions deliberately designed to encourage the growth of *D. lycopersici*. The present field experiments with plants raised from affected seed do not support the view that *Didymella* stem rot is in fact commonly seed transmitted, for in these trials, when plants were raised from samples of seed with either a large or a small proportion of the seeds carrying *D. lycopersici*, losses from stem rot were not significantly greater than among plants raised from seeds that were free of the fungus.

These field experiments were carried out with seeds externally contaminated with naked pycnospores, or with commercial seeds in which the form of the contamination or infection was unknown. Indeed, in the light of the experiments on the longevity of the fungus on seeds, it may be argued that the commercial seed samples used were perhaps quite free of *D. lycopersici* when sown; nevertheless, the degree of infection at sowing time of the artificially inoculated samples was known, and in one case was 100%. Additional trials would be useful with seeds derived from diseased fruit, and showing internal infection with the presence of pycnidia, as described by Fisher (1954), particularly since Liesau (1932) was able to demonstrate that some pycnospores remained alive for 14 months within pycnidia on dried tomato stems. If it is held that, in certain as yet unknown conditions, affected seeds may give rise to diseased plants, the latter

contingency may be guarded against by using seed which is over a year old, as such seed may be expected to be free of *D. lycopersici*.

The experiments reported here, when taken as a whole, suggest that, while *D. lycopersici* is frequently present on newly extracted tomato seed in Jersey, within a year of extraction the seed is free of the living fungus, and further, that the severe and widespread attacks of stem rot experienced in the Island can rarely be attributed to the use of infected seed.

I am much indebted to Dr T. Small, who suggested the present investigations, and who supplied valuable constructive criticism throughout their progress. This paper is based on parts of a thesis submitted for the degree of Ph.D. of the University of London.

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SOIL-BORNE INFECTION OF TOMATOES BY *DIDYMELLA LYCOPERSICI* KLEB.

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Experiments were carried out to examine the effects of contaminated soil on outbreaks of *Didymella* stem rot of outdoor tomatoes in Jersey. In some of these experiments, potted plants were treated with soil from land in which severe attacks of stem rot had occurred, while in others, inoculum was buried in field plots before planting with tomatoes. In the pot experiments, heavy losses resulted when plants were treated with soil which had just carried a diseased crop, but soils tested 5 or more months after the removal of an affected crop caused little or no loss among the test plants. In the field experiments, a large percentage of the plants was destroyed on each occasion when inoculum was buried in the plots in spring, within a few days or weeks of planting. Similar losses resulted in two out of three experiments in which the inoculum was incorporated in the soil in autumn, 6–9 months before planting. It is concluded that if the remains of a crop attacked by *D. lycopersici* are ploughed in, and tomatoes are grown on the land in the following season, *Didymella* stem rot is likely to be severe in the new crop, especially if (as sometimes happens in Jersey), the ploughing-in is left until spring. On the other hand, if the diseased crop residues are removed from the field at the end of the season, the fungal inoculum is greatly reduced by the time the next year's crop is planted, and the danger of disease may then be much decreased.

Introduction

In a previous paper (Phillips, 1956) details were given of the symptoms of *Didymella* stem rot of tomatoes as it affects the outdoor tomato crop in Jersey. Investigations on seed transmission of the disease were then described. The present paper gives an account of experiments on the persistence of *Didymella lycopersici* in field soil. These experiments were carried out to test the commonly held view that primary infection with *Didymella* stem rot, occurring relatively early in the season, and causing disease at or below soil level, is mainly due to attack by the fungus present in the soil (Orth, 1939).

The growth of *D. lycopersici* in soil has been considered by a number of workers. Liesau (1932) grew the fungus in soil under artificial conditions, and found that it grew best on acid soils of pH 5·2. Nevertheless, it grew well over a wide pH range from pH 3·9 to 8·1, although at pH 3·9 growth was slow. In studies on the distribution of the fungus in depth through the soil, Orth (1939) found that it penetrated to a depth of 5 cm. (2 in.) in 20 days, and Sheard (1947) in a similar study recorded penetration to a depth of 3 in. (7·5 cm.) in sterilized soil and $\frac{1}{2}$ in. (1·25 cm.) in unsterilized soil in 6 weeks at 20° C.

Small (1940) concluded that growth through soil was slow, because when he placed cultures and diseased tissue in the soil 1 in. from the foot

of tomato plants, the plants seldom became infected. He carried out a small-scale field experiment, however, in which he buried diseased tomato haulms and fruits the autumn before planting, and he then found that in the plots in which the inoculum was buried, an average of 28 % of his plants showed stem rot at the foot, whereas in his control plot, no plants were diseased. Nevertheless, he remained of the opinion that infection from field soil was probably rare, except when replacing diseased plants (Small, 1943). He considered that propagating soil might be a source of infection if many diseased tomato stems had been added to the compost heap, and if the soil had not then been properly sterilized.

Hickman (1946) also thought that the disease often arose through the use of contaminated propagating soil, and that field soil is probably not often a source of infection. He planted tomatoes in fields which one, two, or three years previously had carried diseased tomato crops, and into which diseased crop residues had been ploughed at the end of the season. His tomato crops remained almost free of stem rot throughout their growth, and he found no disease at the foot of any of the plants.

Orth (1939) was unable to induce the disease by raising plants in either naturally or artificially contaminated soil, and Oyler & Read (1942) found, similarly, that when plants were raised in contaminated soil few became diseased. But Small (1940) found that when he removed diseased plants and replanted immediately, between 28 and 48 % of the replacements contracted disease, while Sheard (1943) successfully infected plants by raising them in artificially inoculated soil.

Direct information on the survival of *D. lycopersici* in soil has been published by Orth (1939) and by Williams (1949, 1950, 1951). The former buried agar cultures during the summer, and obtained some evidence that in his area, in light soils, the summer soil temperatures to a depth of 2 cm. were sufficiently high to kill the fungus. Williams investigated the growth of the fungus under laboratory conditions, and concluded that growth was favoured by high soil moisture content, low temperatures, and smallness of the soil particles. In one experiment (Williams, 1951), he found soil capable of infecting tomato plants 43 weeks after artificial inoculation with *D. lycopersici*.

Experimental

To amplify the results reported in the literature reviewed above, a number of glasshouse and field plot experiments were carried out.

Glasshouse experiments

In three interrelated glasshouse experiments, soil was tested for the presence of *D. lycopersici* by placing it to a depth of approximately half an inch around the bases of tomato plants growing in partially sterilized soil in 6 in. clay pots ('32's'). The soils to be tested were sampled from just below the soil surface, and were mixed with horticultural peat in the proportion three volumes soil to one peat, in order to reduce panning due to watering. The plants, of cultivar Sunrise, were 5–6 in. high when

treated with the soils. After treatment, they were kept in randomized blocks on the benches of a heated glasshouse for 20 weeks, during which period they were frequently examined for the presence of *Didymella* stem rot, which in all the experiments in this series occurred at the foot of the plants only. Diseased plants were removed as soon as they were found. The results of the three experiments are grouped together in Table 1.

Table 1. *Testing for presence of* Didymella lycopersici *in field soil*

Exp.	Time after treatment (days)	Mean percentage plants with stem rot (out of 10 plants for each sub-plot) A	B	C	D	E	F	Least significant differences when P is 0·05	0·01	0·001
1	49	0·25	0	—	—	—	—	—	—	—
	60	0·50	0	—	—	—	—	—	—	—
	86	1·50	0	—	—	—	—	—	—	—
	96	2·00	0	—	—	—	—	—	—	—
	111	2·50	0	—	—	—	—	—	—	—
	115	2·75	0	—	—	—	—	2·72	4·99	—
	118	3·00	0	—	—	—	—	2·91	5·33	—
	128	3·75	0	—	—	—	—	2·72	4·99	—
	133	4·25	0	—	—	—	—	—	3·67	8·13
	140	4·75	0	—	—	—	—	2·72	4·99	—
2	52	0	0	0	0·25	0	—	—	—	—
	59	0	0	0	0·25	0·25	—	—	—	—
	73	0	0	0	0·50	0·25	—	—	—	—
	119	0·25	0	0·25	0·50	0·25	—	—	—	—
	140	0·25	0	0·25	0·50	0·25	—	—	—	—
3	40	0	0	—	—	—	0·40	—	—	—
	48	0	0	—	—	—	0·80	0·70	1·03	—
	53	0	0	—	—	—	1·20	—	1·03	1·54
	62	0	0	—	—	—	1·80	—	1·60	2·40
	70	0	0	—	—	—	2·20	—	—	2·02
	80	0	0	—	—	—	3·20	—	—	1·54
	88, 95	0	0	—	—	—	3·80	—	—	2·02
	105, 126	0	0	—	—	—	4·00	—	—	2·60
	133, 140	0	0	—	—	—	4·40	—	—	2·10

A, C, D, E and F: fields and plots in which heavily diseased tomato crops had been grown. B: control plot in which no tomatoes had been grown. Exp. 1: soils tested at end of season, with diseased crop *in situ* in plot A. Exp. 2: soils tested *c.* 5 months after removal of diseased crops. Exp. 3: soil A tested *c.* 12 months, and soil F immediately after removal of diseased crops.

Experiment 1. In the first experiment, soil was sampled in autumn from plot A, in which tomato haulms attacked by *D. lycopersici* had been buried in the previous May, and in which over 60% of the following tomato crop was destroyed by *Didymella* stem rot at the foot. At the time of sampling on 31 October 1953 (almost 26 weeks after burial of the original inoculum), many diseased plants were still present in the plot. Four replicates of ten plants each were treated with suspected soil, and, as a control, four similar replicates were treated with soil from a field plot B, in which no tomatoes had been grown for at least 15 years. It will be seen from Table 1 that by the end of the experiment, almost 50% of the plants treated with soil from plot A were lost, the difference between the figures for A and B at this stage of the experiment being significant at the 5% level, and approaching significance at the 1% level.

Experiment 2. The second experiment was begun in the same manner on 23 April 1954, approximately 25 weeks after setting up the first, and about 5 months after the removal of the crop residues from plot A. Soil was sampled and tested again from plots A and B, and the experiment was extended to include soils from a further plot C, and two fields, D and E. In the plot C, 50% of the plants of the 1953 tomato crop (removed from the field 5 months prior to the present sampling) were destroyed by stem rot at the foot. In both the fields, the 1953 crop was severely damaged by stem rot, over 90% of the plants in field D being attacked by the end of the season, and the crop in field E being described by the grower as a total loss. Field D was on level ground, field E was on a *côtil* (a steep, well-drained slope). Four replicates of ten plants each were treated with each soil. The figures given in Table 1 show that in this experiment, *Didymella* stem rot caused some very slight loss in each group of plants except the control, but statistical analysis failed to show any significant differences between the various groups of plants. In the case of the plants treated with soil from plot A, the number of plants destroyed by stem rot by the end of this experiment was only one-nineteenth of the number lost by the end of Exp. 1. Five months after the removal of the 1953 tomato crops, therefore, the inoculum in the soil was greatly reduced, and within the statistical limits of the experiment it was impossible to demonstrate the presence of *D. lycopersici* in the soil, even in plot A, which was shown by the first experiment to be heavily contaminated at the end of the cropping season.

Experiment 3. In the third experiment, which was set up on 5 November 1954, soils from plots A and B were sampled and tested again, in comparison with a further plot, F. Plot A had been left fallow since the 1953 crop, and the soil samples were taken approximately 1 year after removing remains of the diseased crop. In plot F, diseased tomato haulms had been buried on 6 April 1954, and about 40% of a tomato crop then planted was destroyed by *Didymella* stem rot at the foot. Sampling was carried out immediately after removal of the diseased crop remains from plot F. The soil from plot F thus corresponded with that from plot A in the first experiment, in that it had just carried a heavily diseased crop, whereas at this stage in the experiments the soil from plot A had carried diseased tomatoes 1 year previously, and in plot B, no tomatoes had been grown for many years. The results of this experiment were similar to those obtained in Exps. 1 and 2, many plants being lost among those treated with soil from plot F, which had borne a heavily diseased crop immediately before testing, but all the plants remaining healthy among those treated with soil sampled 1 year after the removal of the diseased crop, or with soil in which no tomatoes had been grown for a long period of years.

It may be noted that, in these experiments, the disease did not appear until from 40 to 52 days after treatment with the contaminated soil samples, and at this stage very few affected plants were present. The number of diseased plants then rose steadily among the plants treated with soil which had just carried diseased tomatoes.

Field experiments

Experiment 4. To examine the effect of burying diseased crop residues on the incidence of stem rot in the field, Sunrise tomato plants, raised in partially sterilized soil, were planted out in randomized blocks on land that had not been used for tomato growing for at least 15 years. The plots within each block contained forty plants each in two double rows 3 ft. apart; the two rows of each double row were 1 ft. apart from one another, and the plants in the rows were 15 in. apart. The plots measured 12 ft. 3 in. × 8 ft., and were treated as follows:

Series G. The remains of fifty diseased tomato plants showing stem rot lesions were dug into each plot on 16 November 1953, approximately 6 months before the planting of the crop.

Series H. The remains of fifty tomato plants from the same stock as those used for series G, but stored over the winter, were dug into each plot on 6 April 1954, about 6 weeks before planting.

Series K. These plots received no inoculum, and acted as a control.

The plants set out in the above plots were raised from seed that had been tested by plating on agar, and found free of *D. lycopersici*. In two further series of plots, L and M, which, like series K, received no diseased tomato material, plants were set out which had been raised from seed infected or contaminated by *D. lycopersici*. The seed for series L was commercial material containing 1 % affected seeds when tested 5 months before sowing, while that for series M was artificially inoculated, and contained 5 % of affected seeds when sown. Each series was replicated five times. No diseased plants were found in the propagating stages, and the plants were set out in the field on 21 May 1954. After planting, the plots were examined at frequent intervals for the presence of stem rot, and diseased plants were removed when seen, to reduce as much as possible the spread of disease from plant to plant.

The results of the experiment are summarized in Table 2, in which after statistical analysis the mean numbers of diseased plants have been converted to percentages, the least significant difference figures being correspondingly adjusted. In (1) are given the percentages of plants with stem rot at or below ground level, and in (2), the percentages of plants with stem rot either at the foot or elsewhere, because attacks at the foot of the plant are usually regarded as largely primary, and caused by infected soil (Orth, 1939), or infected seed (Hickman, 1946), whereas attacks elsewhere are of secondary origin. It will be seen from Table 2 that the first diseased plants appeared in the soil-inoculated plots, between 4 and 5 weeks after planting. In these plots, the number of diseased plants then rose rapidly to a high level. Disease did not appear in the control series K until 9 weeks after planting. At no time could any significant difference be found between the number of diseased plants in the plots artificially inoculated with *D. lycopersici* 6 months and 6 weeks, respectively, before planting. Comparison of these plots with series K indicates that, as far as attack at the foot of the plants is concerned, the number of plants with stem rot in both the series in inoculated soil was significantly greater than

Table 2. *Effect of buried inoculum on stem rot infection in the field (1954: Experiment 4)*

	Days after planting	Percentage of diseased plants in series					Least significant difference when P is		
		G	H	K	L	M	0·05	0·01	0·001
(1)	32	0	0	0	0	0	—	—	—
	42	0	0·5	0	0	0	—	—	—
	52	1·0	3·0	0	0	0·5	—	—	—
	59	1·0	5·5	0	0	1·0	—	—	—
	63	1·0	8·0	0	0	1·0	—	—	—
	71	6·5	11·5	0·5	0	1·5	—	—	—
	81	15·5	20·5	2·5	3·0	4·0	—	—	—
	89	26·0	24·0	4·0	4·0	5·0	16·30	22·45	—
	95	32·0	34·0	7·5	7·0	6·0	12·10	16·55	22·90
	98	34·0	39·0	14·0	10·5	9·5	12·73	17·53	24·08
	103	35·0	39·0	14·0	11·0	9·5	12·28	16·90	23·25
	115	35·0	39·5	14·5	11·0	9·5	12·00	16·53	22·70
(2)	32	0	0·5	0	0	0	—	—	—
	42	1·0	1·0	0	0	0	—	—	—
	52	2·0	4·0	0	0	0·5	—	—	—
	59	2·5	6·5	0	0	1·0	—	—	—
	63	3·5	10·5	0·5	1·0	1·5	—	—	—
	71	9·5	15·0	1·5	1·5	2·0	—	—	—
	81	24·5	29·0	9·0	11·0	8·0	—	—	—
	89	40·5	35·5	15·0	19·0	14·5	17·70	24·40	33·53
	95	49·5	48·5	23·0	25·0	19·5	14·35	19·75	27·15
	98	60·5	58·0	43·5	37·5	32·0	18·88	26·00	35·73
	103	62·5	59·5	48·5	41·0	35·0	16·60	22·88	31·43
	115	73·0	71·5	69·0	65·5	53·5	—	—	—

(1) Percentage plants with stem rot at foot. (2) Percentage plants with stem rot either at foot or elsewhere. G: inoculum buried *c.* 6 months before planting. H: inoculum buried *c.* 6 weeks before planting. K, L, M: no inoculum buried. G, H, K: plants raised from seed free of *Didymella lycopersici*. L, M: plants raised from seed infected or contaminated by *D. lycopersici*.

the number in the control series K, or in either of the series raised from inoculated or otherwise infected seed. Late in the growing period, secondary spread from plot to plot began to obscure the primary differences between the series, as is shown by the figures based on the total numbers of plants with stem rot, whether at the foot or elsewhere (Table 2 (2)). Nevertheless, 89 and 95 days after planting, these figures also show a significant difference between the number of diseased plants in both series in inoculated soil and the number in the control series K. These results suggest that a detailed study of the spread of *Didymella* stem rot from plant to plant might prove of interest.

Experiments 5 and 6. For these experiments, the remains of tomato crops attacked by *D. lycopersici* were buried in the top soil of small plots at intervals ranging from just over 2 years to 2 days before the planting of the test crop. In one instance, diseased fruits were used as the source of inoculum, but otherwise the inoculum was provided by diseased tomato plants, fifty of which were broken up and buried as uniformly as possible in each plot. The plots measured 11 ft. 3 in. × 12 ft., and were 6 ft. apart. Each plot was planted with fifty tomato plants in five single rows of ten, the rows being 3 ft. apart. The plants, of cultivar Sunrise, were propagated in partially sterilized soil.

The observations were carried out during 1952 and 1953, and the history of the plots for 1952 (Exp. 5) is given in Table 3, while the results for the same year appear in Table 4. In 1952, the five plots, N, O, P, Q and R were planted on 28 May. On that date the remains of the tomato plants buried 9 months previously in plot Q could not be found by macroscopical examination of the soil. Table 4 shows that losses were heavy in plot R, in which inoculum was buried a few weeks before planting, but that few plants were lost in any of the other plots. In this experiment, the first diseased plants were not found until more than 10 weeks after planting.

Table 3. *History of the plots used for Experiment* 5

Plot	Inoculum	Date of inoculum burial	Approx. time between inoculum burial and planting test crop	Percentage of previous crops lost through *Didymella* stem rot
N	Nil	—	—	No previous tomato crop
O	50 diseased plants	7 Mar. 51	15 mth.	1951: 60% (32% at the foot)
P	2 cwt. diseased fruits	31 Aug. 51	9 mth.	No previous tomato crop
Q	50 diseased plants	11 Sept. 51	9 mth.	No previous tomato crop
R	50 diseased plants	6 May 52	3 wk.	No previous tomato crop

Table 4. *Effect of buried inoculum on stem rot infection* (1952: *Experiment* 5)

	Days after planting	Percentage of diseased plants in plot				
		N	O	P	Q	R
(1)	71	0	0	2	0	8
	82	0	0	2	0	12
	86	0	0	4	0	14
	98	0	2	6	0	22
	103	2	6	6	0	36
	112	2	6	6	0	42
(2)	71	0	0	2	0	8
	82	0	0	2	0	12
	86	0	0	4	0	14
	98	0	2	6	0	22
	103	2	6	6	0	38
	112	6	6	12	2	52

(1) Percentage plants with stem rot at foot. (2) Percentage plants with stem rot either at foot or elsewhere.

In 1953, further observations (Exp. 6) were made in the same way on most of these plots, and on two others, S and T, in addition; the plot histories and results, respectively, are given in Tables 5 and 6. Planting took place on 6 May 1953. On that date, when the soil of plot S was examined, the remains of the plants buried in it 8 months previously were found to be still present and only partially rotted. In this experiment, losses were high not only in plot T to which inoculum was added within a few days of planting, but also in plot S into which diseased plants were

dug 8 months earlier. In the other plots, losses were in all cases small until a late stage, though the general level of disease was higher than that obtained in Exp. 5 of 1952.

Table 5. *History of the plots used for Experiment* 6

Plot	Inoculum	Date of inoculum burial	Approx. time between inoculum burial and and planting test crop	Percentage of previous crops lost through *Didymella* stem rot
O	50 diseased plants	7 Mar. 51	2 yr. 2 mth.	1951: 60% (32% at the foot) 1952: 6% (all at the foot)
P	2 cwt. diseased fruits	31 Aug. 51	1 yr. 8 mth.	1952: 12% (6% at the foot)
Q	50 diseased plants	11 Sept. 51	1 yr. 8 mth.	1952: 2% (none at the foot)
R	50 diseased plants	6 May 52	1 yr.	1952: 52% (42% at the foot)
S	50 diseased plants	16 Sept. 52	8 mth.	No previous tomato crop
T	50 diseased plants	4 May 53	2 days	No previous tomato crop

Table 6. *Effect of buried inoculum on stem rot infection* (1953: *Experiment* 6)

	Days after planting	Percentage of diseased plants in plot					
		O	P	Q	R	S	T
(1)	52	0	0	0	0	0	4
	59	0	0	0	0	8	4
	61	0	0	0	0	10	14
	66	0	0	2	0	14	20
	78	16	0	4	6	32	36
	98	20	6	14	6	40	46
	114	20	8	22	8	44	64
(2)	52	0	0	0	0	0	4
	59	0	0	0	0	8	4
	61	0	0	0	0	10	14
	66	0	0	2	0	14	20
	78	18	2	4	8	32	36
	98	24	10	7	8	52	50
	114	38	14	44	22	70	74

(1) Percentage plants with stem rot at foot. (2) Percentage plants with stem rot at foot or elsewhere.

Discussion

In the pot experiments, soils sampled from plots that had just carried a heavily diseased tomato crop caused severe losses among the test plants, but when plants were treated with soils sampled from the same and similar plots, and from fields, 5 months or more after the removal of the crop remains, slight or no loss occurred among them.

The evidence therefore suggests that if the remains of a crop attacked by *D. lycopersici* are removed from the field at the end of the season, the fungus, which is at that time plentiful in the soil, soon disappears, and in 5 months little or none of it may be present.

On the other hand, the field experiments show that the persistence of *D. lycopersici* in the soil is considerably increased if the diseased crop

remains are not removed, but are ploughed into the ground. No complete comparison can be made between the three sets of figures obtained, as the observations were made in three different years, and the severity of any plant disease is affected by weather conditions that vary from season to season. Nevertheless, in all three cases, losses were heavy when inoculum in the form of diseased tomato haulms was added to the soil in the spring, within a few days or weeks of planting time (Tables 2, H; 4, R; 6, T), whereas where the inoculum was incorporated in the ground a year or more before planting, relative losses were very much lower, at least until a late stage in the experiments, when secondary spread of the disease began to take place.

When infected haulms were buried in the autumn before cropping, incubation periods of intermediate length were obtained, and the experimental results were then of particular interest. In two of these field experiments (Exps. 4 and 6), losses were high in the plots inoculated in the previous autumn (Tables 2, G; 6, S). The losses in these cases were similar in magnitude to those in the plots contaminated only a few days or weeks before planting. On the other hand, in Exp. 5, the number of affected plants remained negligible to the end of the observations in plot Q (Table 4), in which diseased haulms affected by stem rot were buried in the autumn, 9 months before the test crop was planted.

In connexion with these results, it is worth noting that in Exp. 5, at planting time no vestiges could be found of the haulms buried 9 months previously, whereas in one of the two experiments in which heavy loss occurred after autumn inoculation of the soil, remains of the buried plants were readily found when planting was carried out 8 months later. Under the edaphic and climatic conditions found in Jersey, organic matter in the soil decomposes rather rapidly. If tomato haulms are buried, it is usually difficult or impossible to find any of their remains 8–9 months later.

At present, *D. lycopersici* appears to show many of the characteristics of the 'root inhabiting fungus' of Garrett (1950). An organism of this ecological type grows actively as a parasite during the life of its host, but after the death of the latter the fungus is less successful as a saprophyte, and undergoes a progressive decline. In the case of *D. lycopersici* incorporated in the soil in dead or moribund host tissues, the rate of this decline may be expected to be influenced by a number of factors, the most important of which are climatic. The stage which the decline will reach at any given time will then be governed not only by these factors, but also by the length of the incubation period. Of the climatic factors, that most likely to affect the longevity of *D. lycopersici* in soil would appear to be the mean soil temperature during the burial period of the inoculum.

Table 7 gives the times of incubation of the autumn inocula and the mean soil temperatures throughout the incubation periods for the experiments referred to above. The table also includes the available information on the visible presence or absence of tomato remains and viability of *D. lycopersici* at the times of planting of the test crops. It will be seen that the period of burial of the inoculum and the mean soil temperature were both greatest in Exp. 5. In this experiment, no tomato remains were found in the soil at planting time, and *D. lycopersici* was then no longer

Table 7. *Effect of time of burial and mean soil temperature on longevity of* Didymella lycopersici *in soil*

Exp. no.	Plot or series	Time of burial (mth.)	Mean soil temp. at 12 in. (° F.)	Presence (+) or absence (−) of haulms at planting time	Presence (+) or absence (−) of *D. lycopersici* at planting time
4	G	6	47·0	−	+
6	S	8	47·1	+	+
5	Q	9	51·5	−	−

present. In the other experiments, in both of which *D. lycopersici* was still present at planting time, incubation time was shorter and soil temperatures were lower than in Exp. 5, and in one of these experiments, the remains of old haulms were still found when the test crop was planted.

These observations suggest the need for further work of a long-term nature to investigate in greater detail the factors influencing the disappearance of *D. lycopersici* from contaminated field soil. Disappearance of the fungus may coincide approximately with the final disappearance of the tomato remains, in Jersey usually from 8–9 months after their burial in the ground. The reason for this, if it should prove to be so, may be indirect only, however, in that factors such as high soil temperature, which favour the destruction of rotting tomato haulms, also accelerate the disappearance of *D. lycopersici.*

The results of these experiments may have some bearing on those of Hickman (1946), who raised a tomato crop with little disease and no basal stem rot in a field in which the previous year's crop had been severely attacked, and into which the diseased crop remains had been ploughed at the end of the season. The time between the clearing of one crop and the planting of another is greater in England than it is in Jersey (where the first plantings may take place in late April, and the last pickings may not be made until November), and the time elapsing between Hickman's two crops may have been just greater than the critical period.

It may be concluded from the results of the experiments here described that if tomatoes are planted in ground that has just carried a crop affected by *Didymella* stem rot, many of them are likely to become infected. This is of significance mainly to those few glasshouse growers who follow a main crop by an autumn planting, and who have no time to carry out any precautions between the removal of one crop and the planting of the next. These results also emphasize the danger of the replacement of diseased plants without an accompanying chemical soil treatment. As far as the outdoor crop is concerned, the experiments suggest that under the conditions found in Jersey, if reasonable hygienic precautions are taken by removing the remains of the crop from the ground at the end of the season, the soil will be free or almost free of *D. lycopersici* by the time the next year's crop is planted, and the danger of disease will be much lessened.

On the other hand, if the remains of a diseased crop are not taken from the field, but are ploughed in, and tomatoes are grown in the land in the following season, heavy losses may be expected in the new crop. It would

appear that if large quantities of inoculum are added to the soil in this way, tomatoes cannot safely be grown on the land in the following year. This is a matter of great importance in Jersey, where many fields carry tomatoes every year. Furthermore, many outdoor growers in the island plough in their old tomato haulms at the end of the season, while others leave the crop remains on the surface throughout the winter, and plough in the haulms in the spring, when preparing the ground for the next crop.

I wish to thank Dr T. Small for much valuable advice freely given. This paper is based on part of a thesis submitted for the degree of Ph.D. of the University of London.

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A NOTE ON TRUFFLE FUNGI IN AUSTRALIA WITH SPECIAL REFERENCE TO *LABYRINTHOMYCES STEENISII* BOEDIJIN

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(With Plate 11)

Records of truffle fungi in Australia suggest their rare occurrence there. However, recent discoveries in New South Wales indicate that they may be more prevalent than was previously suspected. Those recorded in the literature are *Genabea tasmanica* Mass. & Rodw. (*Kew. Bull.* 1898, p. 125), *Genea pazschkei* Bres (*Hedwigia*, 1893, **32**, 118) and *Terfezia tasmanica* Rodw. (*Proc. Roy. Soc. Tasm.* 1925, p. 167), from Tasmania. There is only one record for the mainland of Australia, *Stephensia arenivaga* Cooke & Mass., which was collected by the Elder Expedition of 1891–2. Of these *Genabea tasmanica* will not stand because of nomina confusa (Gilkey, 1954).

Recently two species of truffle fungi were found within fifty yards of each other at Pennant Hills in New South Wales. They were unearthed in virgin bushland soil on Wianamatta shale carrying a canopy of *Eucalyptus pillularis* and *E. saligna*. Three ascocarps of one species were unearthed in 1949 and Dr Gilkey described and named this species *Hydnoplicata whitei* (Gilkey, 1954). In March 1953 a single ascocarp of the other species was dug up amongst many basidiocarps of *Hymenogaster*. This specimen was identified by Dr Gilkey as *Labyrinthomyces steenisii*, described originally by Boedijin in 1939 from Java (*Bull. Jar. Bot. Buitenzorg*, Ser. III, **16**, 238). This specimen is the only other one so far found, apart from the original specimen described by Boedijin and collected by Steenis in 1930 from soil of a terrace wall between cultivated plots on the Dieng plateau in Semarang reserve, Java. The genus *Labyrinthomyces* is considered unique for the Tuberaceae in that exceedingly short paraphyses in the hymenium is a characteristic not known in any other member of the family Tuberaceae.

Boedijin's description does not mention the radiation from the centre of the ascocarp of the labyrinthine chambers which is a distinct feature in the specimen found in Sydney (Pl. 11, fig. 1). This could be missed if a transverse section of the ascocarp was made instead of a longitudinal section. The description of the Sydney specimen is as follows: *Ascocarp* hypogeous, globose, 3·5 cm. in diameter, fleshy, brownish. In section, interior white provided with numerous and elongated labyrinthine-branched chambers radiating from the centre of the ascocarp. *Chambers* 1–10 mm. long, 0·5–2 mm. wide, wholly closed and not opening towards the exterior (Pl. 11, fig. 1), lined with the hymenium. *Hymenium* in regular palisade, consisting of asci and short paraphyses (Pl. 11, fig. 2). *Asci* cylindric, rather thick-walled and mostly variously bent undulating,

attenuated towards the base, 300–335 μ long, 25–32 μ broad, mostly 8-spored (Pl. 11, fig. 3). *Spores* at first irregularly 2-seriate, in mature asci always 1-seriate, globose, tuberculate, pale dirty yellow 19–25 μ in diameter (Pl. 11, fig. 3). *Paraphyses* very short, thread-like, branched at the base, separated, hyaline, sometimes anastomosing, gradually increasing in breadth towards the rounded apex, 90–128 μ long, 5–6 μ broad at the apex.

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EXPLANATION OF PLATE 11

Labyrinthomyces steenisii

Fig. 1. Longitudinal section through two-thirds of the ascocarp, showing the numerous, elongated labyrinthine chambers radiating from the centre. × 6.

Fig. 2. Freehand section through the hymenium and trama of portion of the ascocarp showing the asci and paraphyses. × 75.

Fig. 3. A mature ascus (left) containing eight ascospores with tuberculate walls, appearing as dark spots on the ascospores. An immature ascus (right) showing the sinuous form of the ascus. × 300.

(Photographs by Woodward-Smith)

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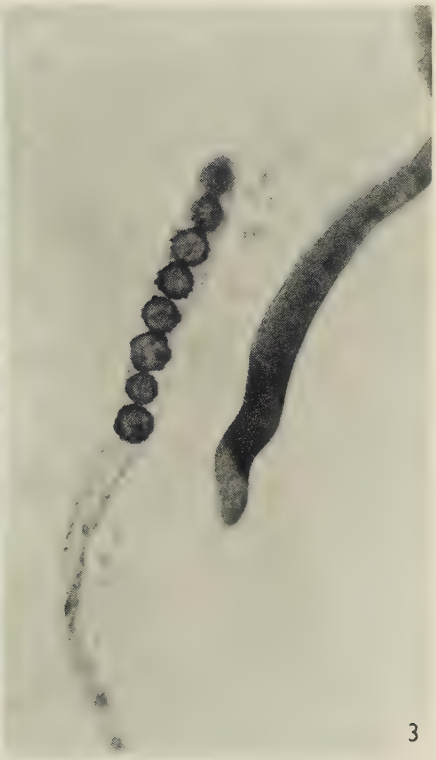

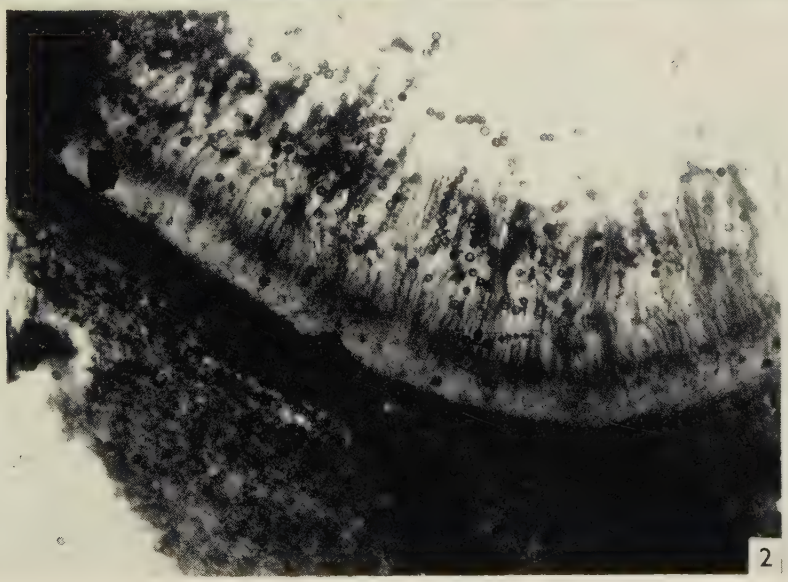

(*Facing p.* 342)

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STUDIES ON *PELLICULARIA FILAMENTOSA* (PAT.) ROGERS

I. FORMATION OF THE PERFECT STAGE

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(With Plate 12 and 3 Text-figures)

Twenty-eight isolates of *Rhizoctonia solani*, from various localities in England and Australia, representing seven pathogenic strains, were induced to form the perfect stage regularly on one or more of three different substrates.

Four of the isolates were identical with *Corticium praticola* Kotila which is renamed *Pellicularia praticola* comb.nov. The remainder were isolates of *P. filamentosa* (Pat.) Rogers, and included apparently non-pathogenic forms as well as a wide range of pathogenic strains. Differences in spore and basidial measurements were as great between isolates within a strain as between different strains. Length of sterigmata varied considerably with different environmental conditions and is of little use for taxonomic purposes.

Introduction

The fungus *Pellicularia filamentosa* (Pat.) Rogers causing stem canker of potato is of world-wide occurrence. It was first described as the sterile mycelium *Rhizoctonia solani* by Kühn in 1858, and it was not until 1903 that Rolfs established the connexion between the vegetative and perfect stages, the latter of which he found occurring on potato stems. Rolfs (1903) sent his material to Burt who identified it as being close to *Corticium vagum*, a saprophyte on pine bark described by Berkeley in 1873, and Burt classified it as a variety of this species, naming it *C. vagum* Berk. & Curt. var. *solani* Burt, because of differences in the number of sterigmata per basidium, and in the size and shape of the basidiospores. Linder (1942), supported by Rogers (1943) and Cunningham (1953), regarded these differences together with the difference in habitat as being important enough to separate Rolfs's material as a different species. However, a basidiospore stage identical with Rolfs's material on potato, and apparently overlooked by Burt, had already been described by Patouillard (1891) on *Dianthus* and *Amaryllus* leaves as *Hypochnus filamentosa*. This preceded by 3 months the description of the perfect state on potato by Prillieux & Delacroix (1891). Rogers (1943) accepted Patouillard's species, but referred it to the genus *Pellicularia* which was established by Cooke in 1876 for *P. koleroga*.

According to Rogers the type material of Patouillard is indistinguishable from the specimens he has examined on potato. This is fortunate as the majority of records of the perfect stage are on potato, and as the organism appears to produce its perfect stage fairly readily on potato, these

fructifications can satisfactorily be used as a reference. Despite the fairly common occurrence of the perfect stage in nature on potato, as yet no reliable method has been devised for inducing isolates obtained during the course of pathological investigations to form this stage under controlled conditions. As a result, most workers have relied on the vegetative and pathogenic characters of the imperfect or *Rhizoctonia solani* stage for identification. Because of the work of Kühn (1858), Prillieux & Delacroix (1891), and Rolfs (1903), the vegative characters of the organism found parasitizing potato have been used as the standard of comparison. On agar it is a mycelium having hyphae 6–12 μ in diameter, without clamp connexions but branching almost at right angles, with septa immediately on either side of the branch, the point of attachment of the branch being constricted, but the base of the branch being swollen and also cut off by a septum. The mycelium is hyaline to light brown in the early stages, later turning darker brown and developing on the surface dark brown sclerotia, irregular in shape, up to 5 mm. across, which may be aggregated in groups.

Isolates possessing these general characters have been shown to differ in details of their morphology and physiology. Morphological variation includes the presence or absence of zonation and colour of the mycelium, hyphal diameter, presence or absence of sclerotia and their size and appearance when present. If grown on the same media under the same conditions these differences are maintained from one transfer to another. Physiological variation includes differences in optimum temperature for growth, ability of isolates to anastomose with each other, ability to use different sources of carbohydrate and enzyme production. Also it has been known for a long time that the pathogenicity of the organism is not confined to potato, but extends over a wide host range. In recent years distinct pathogenic strains have been separated, some of which appear specific to a single host family while others are parasitic on a range of families. The existence of these differences had given rise to speculation that the strains included under *R. solani* may represent more than one species.

This paper records the successful development of the perfect stage for a number of strains differing in pathogenic and vegetative characters, and compares the perfect stages obtained.

Source of isolates used and variation in their pathogenicity

Twenty-eight isolates of *R. solani* obtained from different host plants in several localities (see Table 1) were induced to form the perfect stage. Within these isolates we were able to distinguish seven pathogenic strains by testing all of them on a range of host plants (lettuce, cabbage, radish, kohlrabi, tomato, potato, parsnip, sugar beet, various grasses, cereals and clovers) representing seven host families.

The differences in pathogenicity will be described in a later paper, but they were clear-cut, the seven strains being either non-pathogenic, restricted in pathogenicity either to one or two host families or to the parts of the host they attacked, or having a wider range of hosts.

The isolate Um proved very difficult to maintain in culture. It was

kept growing long enough to carry out a restricted study of its pathogenicity and fructification in soil, but after two subcultures it failed to grow on any one of a range of media tested and the culture was lost. The results with this strain are therefore regarded as tentative.

Variation in vegetative characters

To study variation in hyphal diameter and cultural appearance the different isolates were grown on potato–marmite–dextrose agar* in 9 cm. Petri dishes for 4 days at 25° C.; then 5 mm. circular plugs were cut from the advancing edge of each colony and transferred to three fresh 9 cm.

Table 1. *Source and pathogenicity of isolates induced to fructify*

Isolate	Source	Pathogenic specificity
Co	Lettuce stem, Slough, England	Stems of Compositae and Cruciferae
Cr 1	Cabbage stem, Hitchin, England	Stems of Cruciferae
2	Cabbage stem, Clare, S. Aust.	
So 1	Tomato stem, Cheshunt, England	Stems of Solanaceae
2	Potato stem, Brough, England	
3	Tomato stem, Cheshunt, England	
4	Potato stem, Woodside, S. Aust.	
5	Potato stem, Lobethal, S. Aust.	
6	Potato stem, Balhannah, S. Aust.	
Um	Parsnip stem, Piccadilly, S. Aust.	Stems of Umbelliferae
St 1	Lettuce stem, Slough, England	Stems of all host families tested
2	Clover stem, Keith, S. Aust.	
3	Pearlwort stem, Adelaide, S. Aust.	
4	Pearlwort stem, Adelaide, S. Aust.	
Rt 1	Wheat root, Norfolk, England	Roots of all host families tested
2	Wheat root, Cungena, S. Aust.	
	Oat root, Cungena, S. Aust.	
4	Clover root, Cungena, S. Aust.	
5	Barley root, Keith, S. Aust.	
6	Wheat root, Keith, S. Aust.	
Np 1	Pea stem, Murray Bridge, S. Aust.	Non-pathogenic to all hosts tested
2	Soil Cungena, S. Aust.	
3	Soil Cungena, S. Aust.	
4	Soil Cungena, S. Aust.	
5	Soil Cungena, S. Aust.	
6	Soil Cungena, S. Aust.	
7	Soil Cungena, S. Aust.	
8	Soil Cungena, S. Aust.	

Petri dishes of the same medium. These plates were incubated at 25° C. One of each three was used after 3 days for measuring hyphal diameters, ten measurements being made of the main 'running' hyphae and ten of the short side branches. The remaining two plates of each isolate were used first for measurements of growth rate and then, after incubation for 5 weeks, to obtain colony characters.

All isolates except St 1–4 grew out at first with a hyaline mycelium, began to turn brown after 7–10 days, and then soon developed differences

* Potato–marmite–dextrose agar: 200 g. potatoes finely sliced in 500 ml. of distilled water are kept at 85–90° C. for 30 min. The liquid is filtered off and mixed with another 500 ml. distilled water containing 15 g. agar (previously heated to melt the agar); 20 g. dextrose and 1·0 g. marmite are added and the total volume made up to 1 l. before autoclaving for 20 min. at ½ atm.

in certain characters. Some showed marked zonation in growth while others were uniform; some failed to develop sclerotia, others formed numerous small woolly sclerotia up to 2·0 mm. in diameter, while others again developed hard brown sclerotia varying in size and appearance, either scattered over the surface or aggregated around the original inoculum plug. The differences were constant between the duplicate plates. The isolates differed considerably in their rates of growth under these conditions, varying from 0·25 to 0·45 mm./hr. with an average of 0·33 mm./hr.

Table 2. *Comparing hyphal diameters of main and side branches in the seven strains of* Rhizoctonia solani *grown on potato–marmite–dextrose agar*

	Co		Cr 1–2		So 1–6		Rt 1–6		St 1–4	
Strain	MB	SB	MB	SB	MB	SB	MB	SB	MB	SB
Np 1–8										
MB	N.S.	—	***	—	N.S.	—	***	—	***	—
SB	—	N.S.	—	***	—	N.S.	—	***	—	***
Co										
MB	—	—	N.S.	—	N.S.	—	N.S.	—	***	—
SB	—	—	—	N.S.	—	N.S.	—	N.S.	—	*
Cr 1–2										
MB	—	—	—	—	***	—	N.S.	—	***	—
SB	—	—	—	—	—	***	—	N.S.	—	N.S.
So 1–6										
MB	—	—	—	—	—	—	***	—	***	—
SB	—	—	—	—	—	—	—	***	—	***
Rt 1–6										
MB	—	—	—	—	—	—	—	—	***	—
SB	—	—	—	—	—	—	—	—	—	***

N.S., not significantly different.
* Significantly different at 5% level.
** Significantly different at 1% level.
*** Significantly different at 0·1% level.

Isolates St 1–4 were distinct in both cultural characters and growth rate. They formed a dense mealy greyish-white surface growth which did not change colour even after 6 weeks. Numerous discrete purplish-brown sclerotia up to 1·0 mm. in diameter and arranged in a zonate pattern developed within the agar and on the glass sides and lid of the Petri dish, but there were none on the agar surface. The rate of growth of these isolates varied from 0·52 to 0·65 mm./hr. with an average of 0·55 mm./hr. These differences in cultural appearance and growth rate enabled isolates St 1–4 to be readily distinguished from all other isolates after 48–72 hr. in culture. These hyphal diameter measurements were analysed statistically and the results, showing whether differences were significant or not, are set out in Table 2.

Development of perfect stage

The isolates were induced to fructify on one or more of three different substrates. Two of these were natural substrates: the surface of inoculated soil, and the stems of plants growing in inoculated soil, both being set up

in open glass jars in the greenhouse. The third substrate was the surface of agar in Petri dishes incubated in the laboratory.

The most satisfactory method for developing fructifications on the natural substrates was to inoculate a maizemeal-sand culture of each isolate at the rate of 2·5 % into unsterilized Waite Institute soil.* Wheat seeds, tomato seeds, or sprouted potato cuttings were planted in this inoculated soil in glass jars 9 cm. in diameter and 9 cm. high, which were then placed in the glasshouse. The soil moisture level was maintained near to 18 % by daily watering.

For developing fructifications on the surface of agar in Petri dishes, the most satisfactory method was to incubate the isolate on potato–marmite–dextrose agar at 25° C. for 7–8 days, and then inoculate it on to soil-extract agar†; after a further 7 days incubation at 25° C. the Petri dishes were removed from the incubator to a laboratory bench.

(1) *Fructifications on the soil surface*

In all cases the hymenium consisted of a tenuous, mealy to powdery, whitish grey layer. It appeared from 5 to 21 days after inoculation of the pots, developed its greatest intensity 5–10 days after it first appeared and lasted a further 5–7 days. Towards the end of this time it gradually turned brown and disappeared. It seemed to develop best when the surface of the soil was very uneven, or lumpy, the lumps being firm aggregations up to 1·5 cm. in diameter. The hymenial layer frequently formed as strongly on the underside of the lumps as on the upper side. It was rarely produced a second time on pots maintained for several months.

No exhaustive examination of the factors controlling initiation of the perfect stage was attempted but those which consistently influenced it are set out below:

(i) The fructifications developed equally well with or without plants in the soil.

(ii) Fructifications were slightly reduced in abundance when the rate of maizemeal-sand inoculum was increased from 2·5 to 5 %, and were further reduced if the soil was inoculated only with a suspension of mycelium in distilled water.

(iii) Fructifications failed to develop when Waite Institute soil had been pretreated either by autoclaving for 1 hr. at one atmosphere or by adding Krillium at the rate of 1:300, or when West Coast soil‡ was substituted.

(iv) Factors such as light and temperature did not appear to be critical. Fructifications developed under glasshouse conditions in both summer

* Waite Institute soil is a heavy red-brown silt loam; wilting point, 6–8 % moisture; field capacity, 20–22 % moisture; pH 6·5.

† Soil-extract agar was made up by autoclaving 1 kg. air-dried soil with 1 l. distilled water for 30 min. at 1 atm. After standing overnight, the supernatant liquid was filtered through filter-paper pulp. To this was added 1·0 g. dextrose, 0·1 g. yeast extract, 0·2 g. KH_2PO_4 and 20 g. agar. The liquid was made up to 1 l., adjusted to pH 7·0 and sterilized.

‡ The West Coast soil was from Cungena, South Australia; it is a dull brown, calcareous sandy loam; wilting point, 5·7 %; field capacity, 20 %; pH 8·9.

(average max. temp. 95° F., average min. temp. 65° F.) and winter (average max. temp. 65° F., average min. temp. 48° F.) and also in pots held at a fairly constant temperature of 75° F. in Wisconsin tanks. Similarly, with regard to light, the hymenium was formed on soil in pots under light intensities varying from 200 to 300 up to 2000 foot-candles.

Strain Rt 2 fruited prolifically on the surface of soil inoculated with cornmeal-sand at the rate of 5%, for a field experiment at the Waite Institute.

(2) *Fructifications on plant stems*

The hymenium developed as a tenuous, mealy to powdery, whitish-grey layer adhering closely to the plant stem and extending from soil level to 4 or 5 cm. above this. Though it took longer to appear (28–35 days after inoculation) it lasted the same period as when formed on soil, and reacted similarly to light and temperature. Examination of epidermal strips carrying the hymenium showed that the fungus was entirely external, but in the strain So 1–6, as the hymenium began to degenerate, the epidermal cells of the potato and tomato stems were penetrated by the hyphae, producing a browning of the stem.

(3) *Fructifications on the surface of agar*

All isolates in the strains Np 1–8 and St 1–4 fruited on the surface of the agar. In both groups the hymenium consisted of small plaques up to 1·0 mm. in diameter scattered over the plate with a tendency, especially in the isolates St 1–4, to form more freely in the outer 2 cm. of the plate. The plaques were at first mealy, white and flat, appearing 1–10 days after the cultures had been removed from the incubator and placed on the laboratory bench. The isolates St 1–4 retained this appearance, but in the group Np 1–8 during the 7–10 days after first appearing the plaques thickened to a spherical outline, and became light brown in colour, resembling small sclerotia. See Pl. 12, fig. 1.

During the 10 days after first appearance spores were freely produced, but after this period the basidia gradually collapsed and spore production ceased. Eventually, about 28–35 days after their first appearance, the cells of the original plaque became empty of protoplasm and there was little macroscopic trace of them, although under the microscope the cell walls remained to give an outline of the original hymenium. In early microscopic examination it was seen that these plaques tended to form along the length of certain hyphae only. These were indistinguishable morphologically from any other hyphae on the plates, but if they formed plaques near the perimeter of the plate further plaques formed freely on the same hyphae towards the centre of the plate, whereas closely neighbouring hyphae did not form plaques anywhere along their length.

Again no detailed examination of the factors influencing the initiation of the hymenium was attempted, but the following factors appeared to be important.

(i) Fructifications failed to develop on nutrient-rich media like potato–marmite–dextrose agar, but developed more freely on soil-extract agar if

the isolate had been previously grown for 7–8 days at 25° C. on potato–marmite–dextrose agar.

(ii) Incubation for longer than 7–8 days at 25° C. on soil-extract agar delayed and reduced or even prevented the hymenium from developing.

(iii) After removal from the incubator, hymenial development was more prolific under reduced light in a corner of the laboratory than in well lit areas near windows.

(iv) Differences in day temperature within the range 60–85° F. apparently had no marked influence, although best development seemed to occur when there was a marked difference between day and night temperatures.

Comparison of perfect stages

The twenty-eight isolates differed in their ability to form a hymenium on the three substrates described, but over a series of experiments the results were consistent, and are set out in Table 3.

Table 3. *Substrates on which twenty-eight isolates of* Rhizoctonia solani *developed the perfect stage*

	Substrate		
Isolate	Soil surface	Base of plant stems	Agar surface
Co, Rt 1–6	Profuse development	None	None
Cr 1–2	Occasional flecks, 1·0 mm.[2]	Profuse development on wheat, potato, tomato	None
So 1–6	None	Profuse development on potato, tomato	None
Um	None	Profuse development on parsnip	None
St 1–4	None	None	Sparse to profuse
Np 1–8	Profuse development	None	Profuse development

Isolate Um was the only isolate tested on parsnip as it was found fructifying under natural conditions on this host and failed to fructify on any other substrate in this experimental work.

For microscopic examination and camera lucida drawings areas of hymenium approximately 1 mm. square were mounted in tap water. To standardize measurements, basidia were measured only when sterigmata were well developed, and sterigmata were measured only when spores were developed to more than one-third the final size. During the mounting, immature basidia which might be confused with spores, being similar in shape but slightly larger, often became detached, and so a piece of undisturbed hymenium was mounted over agar and spores shed on to the agar surface were measured.

At least ten measurements each of basidia, sterigmata and spores were made for each isolate, including measurements for each substrate on which the isolate fructified. These measurements, together with the results of a statistical analysis, are set out below in Tables 4 and 5. In the tables the isolates within each pathogenic strain are subdivided according to their geographical origin, to determine whether there were any differences relating to their occurrence in separate localities.

Table 4. *Measurements (μ) of basidia, sterigmata and spores for different strains*

Isolate	Origin	Substrate	Basidia	Sterigmata	Spores
Np 1	Murray Bridge, S. Aust.	Soil	12·9–15·9–18·3 8·8– 9·4–10·4	10·4–16·8–20·8 2·9– 3·0– 3·1	8·1–8·5– 9·0 5·0–5·6– 6·0
		Agar	13·6–15·2–18·3 8·3– 9·4–10·4	12·5–21·5–36·5 2·7– 2·9– 3·1	8·1–8·6– 9·2 4·8–5·4– 6·0
Np 2–8	Cungena, S. Aust.	Soil	13·5–17·6–21·8 7·9– 9·4–10·8	13·6–16·5–22·8 2·9– 3·1– 3·5	7·9–9·0–10·6 4·8–5·4– 6·5
		Agar	14·2–16·6–21·4 7·9– 9·4–11·2	8·5–20·4–46·8 2·7– 3·2– 3·8	7·1–9·0–10·5 5·0–6·0– 6·7
Co	England	Soil	15·0–16·8–18·3 8·3– 9·2–10·4	12·7–15·1–18·7 2·5– 2·8– 3·1	8·1–8·5– 8·8 5·8–6·2– 6·5
Cr 1	England	Plant stem	15·0–16·9–18·3 8·3– 8·6– 9·3	13·5–16·2–18·5 2·5– 2·9– 3·1	7·9–9·1–10·4 5·2–5·7– 6·5
Cr 2	S. Aust.	Plant stem	15·0–16·8–17·3 8·3– 8·6– 9·4	13·5–15·8–20·4 2·5– 2·8– 2·9	7·9–9·1–10·4 5·2–6·0– 6·4
So 1–3	England	Plant stem	12·4–16·3–19·2 7·5– 9·1–10·6	10·4–13·7–21·4 2·5– 2·8– 3·1	7·9–9·0–10·4 5·0–6·1– 6·7
So 4–6	S. Aust.	Plant stem	14·5–16·4–18·7 8·3– 8·9–10·8	13·5–16·2–19·8 2·7– 3·0– 3·2	8·3–9·6–10·4 6·0–6·4– 6·7
Um	S. Aust.	Plant stem	12·4–14·0–15·2 7·5– 8·8–10·0	8·3–10·1–12·5 2·5– 2·8– 2·9	7·7–8·3– 8·8 4·6–5·1– 5·8
St 1	England	Agar	15·6–17·6–21·2 7·5– 8·3– 8·7	17·5–23·6–33·7 2·5– 2·6– 2·9	7·3–9·2–10·4 5·4–6·1– 6·5
St 2	Keith, S. Aust.	Agar	14·6–17·1–18·7 6·7– 7·5– 8·3	15·6–28·4–43·7 2·7– 2·9– 3·5	8·3–9·2–10·8 6·2–6·4– 6·7
St 3–4	Adelaide, S. Aust.	Agar	15·6–17·4–18·7 7·7– 8·1– 8·3	10·4–13·5–15·6 2·7– 2·8– 2·9	8·5–9·6–10·0 6·2–6·6– 7·5
Rt 1	England	Soil	15·2–16·6–17·6 8·7– 9·0– 9·4	13·9–15·2–16·6 2·5– 2·6– 2·9	8·3–8·5– 8·9 6·0–6·2– 6·5
Rt 2–4	Cungena, S. Aust.	Soil	12·4–16·1–21·8 7·9– 9·0–11·2	14·4–16·8–18·7 2·5– 3·0– 3·8	7·7–8·9–10·4 4·8–6·1– 6·5
Rt 5–6	Keith, S. Aust.	Soil	14·2–17·0–25·2 7·5– 9·0–14·6	13·6–16·3–18·9 2·5– 2·8– 4·2	8·3–9·1–10·4 5·8–6·2– 6·7
Rogers' (1943) limits			(10–)12–18(–23) 8–11(–12·5)	3–8·5–20 1·5–3·5–4·5	7–12·5 4–7

Upper line, length of structure; lower line, breadth of structure.

Table 5. *Statistical comparison of basidia and basidiospore measurements for different strains of* Rhizoctonia solani *from various localities*

	Basidia		Basidiospores	
Comparison	Length	Breadth	Length	Breadth
St 1–4 *vs.* all others	*	***	***	***
Between others	***	**	**	***
Np 1 *vs.* Np 2–8	**	N.S.	*	**
Between Np 2–8	*	N.S.	N.S.	N.S.
Cr 1 *vs.* Cr 2	N.S.	N.S.	N.S.	*
So 1–3 *vs.* So 4–6	N.S.	N.S.	**	N.S.
Between So 1–3	N.S.	**	N.S.	N.S.
Rt 1 *vs.* Rt 2–6	N.S.	N.S.	N.S.	N.S.
Rt 5–6 *vs.* Rt 2–4	*	N.S.	***	*
Rt 5 *vs.* Rt 6	N.S.	***	N.S.	N.S.
Between Rt 2–4	**	*	***	***
St 1 *vs.* St 2–4	N.S.	N.S.	N.S.	*
St 2 *vs.* St 3–4	N.S.	N.S.	N.S.	N.S.

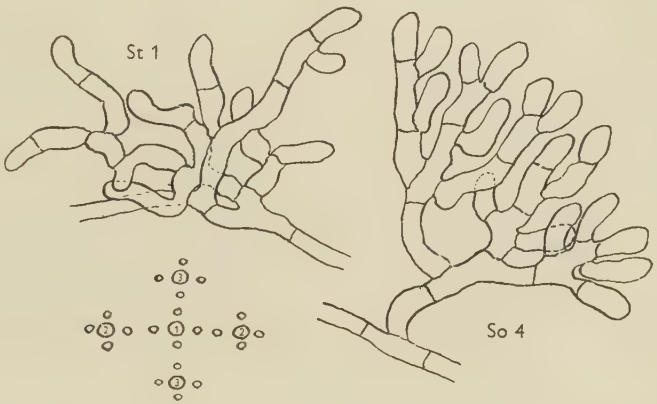
N.S., not significantly different.
* Significant difference at 5 % level.
** Significant difference at 1 % level.
*** Significant difference at 0·1 % level.

Isolates of pathogenic strain St 1–4 appeared to differ from all other isolates in three characters.

(i) The branching of cells immediately prior to basidial formation was regularly cymose in all other isolates resulting in small tightly packed spherical aggregates of basidia, but in St 1–4 the early branching was racemose in character and much less regular and there was no definite resulting pattern of basidia, the aggregates being loose flat plaques rather than closely spherical.

(ii) Spore and basidial measurements were significantly different at the 0·1 % level from the other strains considered as one group. Both spores and basidia were longer and thinner in St 1–4 than in the remaining isolates.

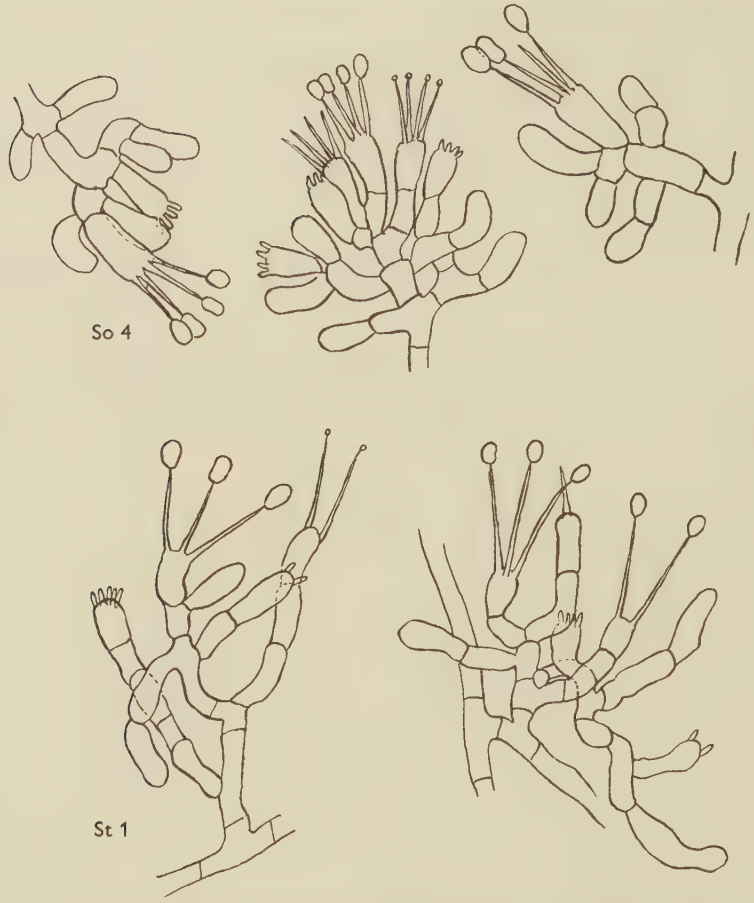
(iii) The number of sterigmata in St 1–4 isolates varied from one to five with an average of three, whereas in all other isolates it was regularly four but rarely three or five per basidium. These characters are illustrated in Pl. 1, figs. 2–5, and Text-figs. 1 and 2.



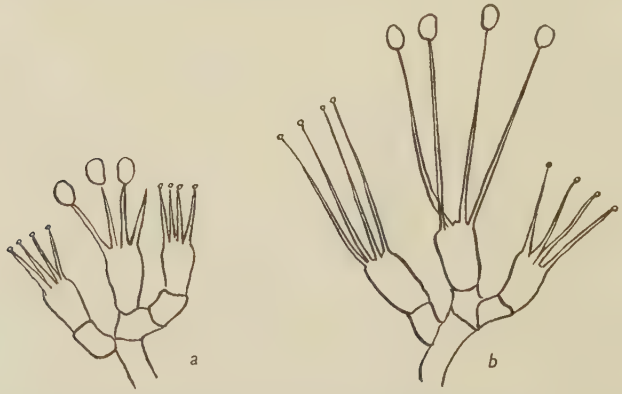
Text-fig. 1. Camera lucida drawings and diagrammatic representation of the branching leading to basidial formation in the two types respresented by St 1 and So 4.

The only differences observed between the fructifications of isolates St 1–4 were that whereas St 1–2 fruited freely over the outer 2–3 cm. perimeter of the plates, isolates St 3–4 produced only occasional flecks (two to twenty per plate) of hymenium. These developed more slowly in the latter isolates, appearing only after 2–3 weeks and persisted much longer, still forming spores 5–6 weeks after they first appeared. These differences were repeated in each batch of plates that were incubated.

Excluding St 1–4, all other isolates appeared identical in microscopic characters with a regular cymose branching of cells leading to a definite pattern in basidial formation of spherical clumps of tightly packed basidia. Four sterigmata, rarely three or five, developed per basidium. The appearance of the perfect stage is illustrated in Pl. 12, figs. 2–5, and Text-figs. 1–3. The measurements for spores and basidia vary considerably, but they all fall within the limits accepted by Rogers (1943) for *Pellicularia filamentosa*. A statistical analysis of these measurements showed significant



Text-fig. 2. Camera lucida drawings of two types of the perfect stage illustrated by So 4 and St 1.



Text-fig. 3. Camera lucida drawings of Np 5 illustrating the variation in length of sterigmata on soil (*a*) and on agar (*b*).

differences between the pathogenic strains. However, a comparison between isolates within strains showed significant differences between isolates from different areas and significant differences between the three isolates Rt 2–3–4 from one locality at Cungena, South Australia. The measurements for these three isolates are set out separately in Table 6.

Table 6. *Basidia and spore measurements (μ) for isolates Rt 2–3–4 from Cungena, South Australia*

Strain	Basidia	Basidiospores
Rt 2	13·6–16·9–19·8	8·1–8·7–10·2
	7·3– 8·5– 9·4	5·6–6·2– 6·4
Rt 3	12·5–14·6–16·6	7·7–8·9–10·2
	8·7– 9·3–10·4	5·8–6·2– 6·4
Rt 4	14·1–16·4–17·3	7·3–7·8– 8·4
	7·9– 8·6–10·4	5·2–5·6– 5·8

The length of the sterigmata has been used in isolates of *P. filamentosa* as a distinguishing character. In isolates Np 1–8, which fruited both on soil and on agar, the sterigmata were much more variable but significantly longer on agar. Humidity appeared to be an important factor influencing length. After the hymenium had developed, moist filter-papers were placed inside the lids of the Petri dishes for 24 hr. Examination of these plates showed that the sterigmata had grown much longer than in plates with no filter-paper, and often the sterigmata continued to develop on the agar as ordinary hyphae, without producing spores at all.

In all isolates which fructified on agar, Np 1–8 and St 1–4, spores frequently germinated by repetition, producing a structure closely resembling a sterigma, about 15 μ long at the apiculus and forming a second spore approximately the same size as the original one. Other spores germinated directly by a germ tube.

Discussion

All isolates other than St 1–4 were identified as *P. filamentosa*. Isolates St 1–4 agreed closely with *Corticium praticola* Kotila and, although the basidia and basidiospore measurements are slightly greater than those quoted by Kotila (1929), it is suggested that isolates St 1–4 were the same as Kotila's organism. The taxonomic position of *C. praticola* is not clear: Hawn & Vanterpool (1953) accept it as a separate species, Rogers (1943) regarded it as a species inquirendae pending further information, while Cunningham (1953) listed it as a synonym of *Pellicularia filamentosa*. The author considers the differences shown by other workers and those outlined in the above investigation are sufficient to retain the original separation made by Kotila (1929), although further work on the variation of the two species, especially by a study of single basidiospore isolates, may necessitate a revision. Following the work of Rogers (1943), however, it appears that the organism belongs in the genus *Pellicularia* and the author proposes to reclassify it as **Pellicularia praticola** comb.nov.

(*Corticium praticola* Kotila, *Phytopathology*, **19**, 1059, 1929). The important differences between *P. praticola* and *P. filamentosa* are set out below:

Pellicularia filamentosa	*Pellicularia praticola*
Four sterigmata per basidium, rarely three or five	One to five sterigmata per basidium, most commonly three
Basidia formed in tight clusters on imperfectly symmetrical cymes	Basidia in loosely arranged flat plaques following irregular racemose branching
Basidia, 12–18 × 8–11 μ	Basidia, 14·5–21·5 × 6·5–8·7 μ
Basidiospores, 7–12·5 × 4–7 μ	Basidiospores, 7–10·8 × 5·5–7·5 μ
Main branch hyphae, 7–12·5 μ	Main branch hyphae, 7–10 μ
Side branch hyphae, 8–16·5 μ	Side branch hyphae, 8–12 μ

In addition to these morphological characters, the faster growth rate of *P. praticola* and its cultural appearance on potato–marmite–dextrose agar as previously described enable it to be distinguished readily from *P. filamentosa* in the absence of the perfect stage.

Kotila (1929) emphasized the importance of length of sterigmata as a distinguishing character but, as shown above for one strain of *P. filamentosa*, the average length can be increased from 16·8 to 21·5 μ and maximum length from 22·8 to 46·8 μ by using different substrates; further increases can be obtained by raising the humidity. It is likely that such variation could be obtained in *P. praticola* if it were induced to fructify on different substrates, and therefore sterigmata length appears to be of little use as a distinguishing character.

If this variability in length of sterigmata is understood, then the organism described from flax by Hawn & Vanterpool (1953) also agrees closely with *P. praticola*. These workers state that there were commonly four sterigmata per basidium, but their figures illustrate as many basidia with two or three as with four sterigmata, and this was not found by the author to be the case with *P. filamentosa*.

P. praticola appears to be a widespread and important pathogen, but in the absence of the perfect stage it has usually been identified as *P. filamentosa*. Flentje (1952) recorded for the first time its occurrence in several areas in England and South Australia, and it has since been identified by the author as an important pathogen in Victoria and New South Wales. Kerr (private communication) has shown that it causes brown patch in lawns in South Australia. The author also agrees with Vanterpool (1953) that Houston's (1945) type A strains, although not induced to form the perfect stage, are probably *P. praticola*.

All isolates other than St 1–4 were identified as *P. filamentosa*, and were similar in the general structure of the perfect stage, the only differences being the dimensions of basidia and basidiospores. Differences in these dimensions were in some cases significant between different strains, but this is of doubtful importance in view of the fact that within one pathogenic strain the three isolates Rt 2–3–4 from one area were identical in cultural appearance, but differed significantly in basidial and basidiospore dimensions. They also differed significantly in hyphal diameter measurements. Alternatively, the isolates So 1–6, which showed no consistent significant

difference in perfect stage or hyphal diameter measurements, differed markedly in their cultural appearance.

These results agree with the tentative conclusion of Rogers that, in respect to morphological and cultural characters, *P. filamentosa* must be regarded as a rather variable species. The author agrees with the ranges given by Rogers (1943) for basidia and basidiospore measurements, but in the isolates examined above the hyphal diameters varied from 6·9 to 16·2 μ, as compared with the range 4·5–14 (–17) μ quoted by Rogers (1943). There was a much smaller variation in diameter of the main running hyphae, 6·9–12·5 μ, than in the short side branches, 8·3–16·2 μ, and it may be more satisfactory to state hyphal diameters specifically in terms of the type of hypha rather than to give general measurements. The number of sterigmata per basidium is regularly four, rarely three or five.

The author also agrees with Rogers (1943) that *P. filamentosa* includes a wide range of pathogenic strains. Six distinct strains were included in the above study. However, *P. filamentosa* is generally regarded as a pathogenic species, a character regarded by Rogers (1943) as apparently reliable. Isolates Np 1–8 have proved non-pathogenic to a wide range of hosts tested at different temperatures. Isolates of this type are common in at least two soils in South Australia, and the range of hosts tested corresponds to that growing in appreciable numbers either as wild or cultivated plants in these soils. These isolates Np 1–8 may prove to be pathogenic to other plants, but at present it would be best to regard *P. filamentosa* as containing non-pathogenic forms which occur in nature as soil-borne saprophytes.

The isolates which developed their perfect stage on natural substrates did so quite readily, and in exactly the same location in each experiment under the conditions described. Those which fruited on the soil surface did so irrespective of whether or not plants were growing in the pots. Factors which influence the initiation of the hymenium must be rather critical, as fruiting was entirely suppressed when a lighter sandy soil was used instead of the Waite loam, or when the Waite loam was treated by autoclaving or by the addition of Krillium. On agar the results emphasized the importance of environmental factors. Five replicate plates were usually inoculated with the one isolate, the agar having been dispensed from one bulk source. In two plates side by side on a bench, one would sometimes show a profuse development of hymenium whereas the second would show none or a few small flecks. Although all isolates have not yet been induced to fruit on agar, they have been induced to do so regularly on natural substrates, and further investigation of the factors influencing this development may lead to a suitable technique for the growth and study of the perfect stage on artificial media under controlled conditions.

I wish to thank Dr C. G. Hansford for advice on the taxonomic aspects, Mrs I. Mathison for statistical analyses and Mr K. P. Phillips for the photography.

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EXPLANATION OF PLATE 12

Fig. 1. Fructifications of isolates Np 5 (left) and St 1 (right) on soil agar 5–7 days after first appearance.

Fig. 2. Np 5. Basidiospores shed on to the agar from the tight spherical cluster of basidia.

Fig. 3. St 1, showing the loose irregular type of branching leading to formation of basidia.

Fig. 4. St 1. Irregular pattern of basidia, each with two or three sterigmata.

Fig. 5. St 1. Basidiospores shed onto the agar and germinating by repetition or by germ tubes.

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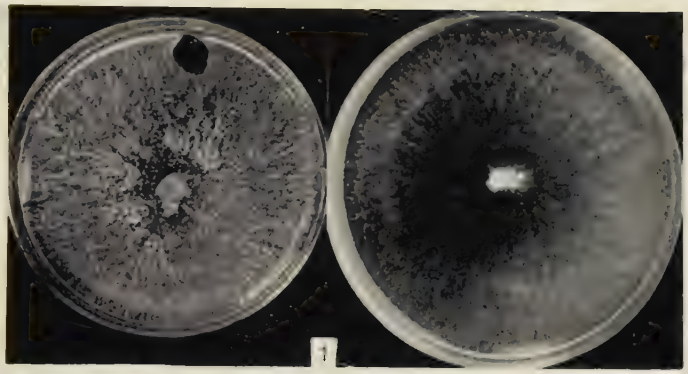

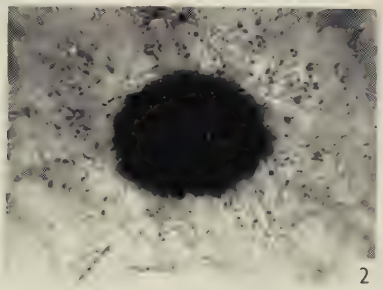

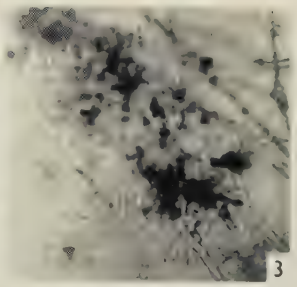

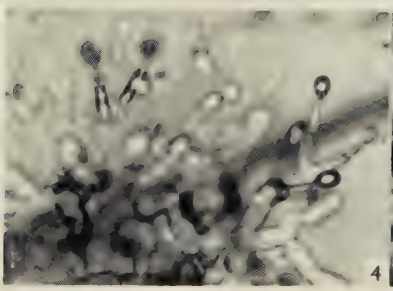

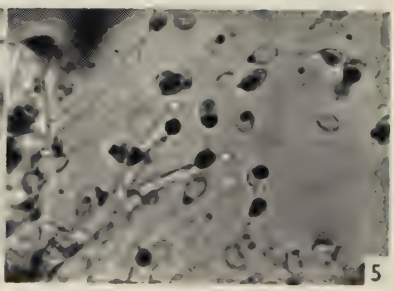

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A PRELIMINARY SURVEY OF FUNGI IN SOME SUDAN SOILS

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This was the first attempt in the Sudan to investigate the microscopic fungi inhabiting the soil. The Waksman dilution method, Warcup's soil-plate method and Chesters's immersion-tube technique were used for ten soil samples from the top 6 in. at ten sites. A list of the isolated fungi is given. Few qualitative differences were observed between the species of fungi found in different soils.

Clouston (1935–40) isolated some fungi from roots in the Gezira soil but no other records have been found. It has therefore been imperative to aim first at finding which fungi are present rather than which are the more active in the soil. The Waksman dilution method (1922), as recommended by Brierley (1923), was therefore chiefly used, but the Warcup (1950) and Chesters (1948) techniques were also tried.

For Waksman's method, five culture media were used, namely Waksman's, modified Waksman (dextrose, 20 g.; asparagin, 2 g.; $MgSO_4 . 7H_2O$, 0·5 g.; $FeCl_3$, 0·1 g.; NaCl, 0·5 g.; and agar 20 g.; distilled water 1000 ml.), Conn's, Czapek-Dox and soil-extract agar, at incubation temperatures varying over the range 29–34° C. The fungi that appeared were more or less the same on all media. However, overgrowth of *Rhizopus* in the first two rendered them less suitable for the dilution method. By comparison, the other three media are less rich, and therefore have proved more suitable, particularly when acidified or used with rose bengal. Acidification of the media to a pH 4·5–5 was found necessary to check the overwhelming growth of bacteria which appeared at pH 5·5 or more. Rose bengal at 1:15,000 was not as effective in its suppression of the bacteria or of *Rhizopus* as was found by Dawson & Dawson (1947).

There was no significant difference, however, between media made up with rose bengal, at 1:15,000 or 1:10,000, and acidified media in the numbers and types of fungi growing on them. Rose bengal considerably limited the growth in diameter of all the fungi, and particularly so at 1:5000. Inoculations of bacteria, *R. stolonifer* and other fungi were made on all media, for comparison with the findings from soil dilution plates, under the same conditions.

The directives in Brierley, Jewson & Brierley (1927) were followed. Soils from ten different sites were sampled from the top 6 in., and their moisture and clay contents were determined. All samples came from heavy sodium-alkaline clay soils from a locality some 7 miles north of Khartoum on the east bank of the Nile, except one less alkaline silt soil from a site near the river bank.

The soils in the order examined were:

Soil I. Rich in organic debris. The sample was obtained from a plot containing the crowns and roots of cut dura hay (*Sorghum vulgare*). Collected in August. Clay content 30%. Moisture content 10·5.

Soil II. From same plot as I above, collected 4 weeks later. Clay content 30%. Moisture content 6·8.

Soil III. Same plot as I; collected 12 weeks later. Clay content 30%. Moisture content 6·3.

Soil IV. Virgin soil. Vegetation sparse; fairly abundant only during the rainy season (July to September); sample collected in November. Clay content 20%. Moisture content 3·8.

Soil V. Same as IV above, collected in February. Clay content 15%. Moisture content 3·7.

Soil VI. River silt, collected 2 ft. from the edge of the Nile in October, before the typical 'gerf' vegetation had started. Clay content 15%. Moisture content 18·5.

Soil VII. Same as VI above, collected 8 weeks later; 'gerf' vegetation present. Clay content 15%. Moisture content 4·9.

Soil VIII. Under *Medicago sativa*, collected in July. Clay content 35%. Moisture content 17·2.

Soil IX. Same as VIII above, collected 6 weeks later. Clay content 35%. Moisture content 10·5.

Soil X. Fallow for 6 months succeeding crop of *Medicago sativa*, collected in June. Clay content 35%. Moisture content 6.5.

Duplicate plates were used for soil dilutions of 1:20,000, 1:40,000 and 1:100,000, in order to obtain a suitable dilution for a counting and examination. Counts were made on the third and fourth days. Sporulation occurred in most cases on the third day; complete overcrowding of colonies occurred on the fifth day, and by the eighth day the perithecial state of some Aspergilli, e.g. *Aspergillus nidulans*, completely replaced the conidial state. The numbers of fungi per gram of soil were consistently too high to be anything but largely a development of colonies from spores.

Growth appeared in less than 36 hr. on all media; bacterial growth predominated within 3 days at the natural pH values of the media. Acidification to pH 4·5–5 was therefore necessary, using sulphuric acid with bromocresol green indicator and a 'Lovibond' comparator. The fungi grew well on all media and produced asexual spores on the third day, covering the plates completely by then, where rose bengal was not used.

For the Chesters immersion tube and the Warcup soil-plate technique, plain agar (instead of nutrient agar as in Warcup, 1950) was used.

Rhizopus stolonifer, *Aspergillus nidulans*, *A. niger*, *A. flavus*, *Curvularia lunata*, *Alternaria tenuis*, and *Fusarium solani* have been the most frequent fungi isolated by these three methods. They have appeared in most soils (see Table 1); they were the dominant isolates not only with the Waksman method, where they may have been largely represented by their spores (Conn, 1918; McLennan, 1928), but also when the other two methods with plain agar were used.

Table 1. *Occurrence of fungi in samples of Sudan soil*

Name of organism	(For details see text) I	II	III	IV	V	VI	VII	VIII	IX	X
Pythium aphanidermatum (Edson) Fitz.	×	×	×	–	–	–	–	×	×	×
Pythium sp.	–	×	–	–	–	–	–	–	–	–
Absidia blakesleeana Lendn.	×	×	–	–	–	–	×	–	–	–
Rhizopus stolonifer (Ehrenb. ex Fr.) Lind	×	×	×	×	×	×	×	×	×	×
R. arrhizus Fischer (syn. *R. nodosus*)	×	×	×	–	–	–	×	×	×	–
Mucor hiemalis Wehmer	–	×	×	–	–	–	×	–	–	–
M. circinelloides van Tiegh.	–	–	×	–	–	–	×	–	–	–
Thamnidium (? *elegans*)	×	×	×	–	–	–	–	–	×	×
Syncephalastrum racemosum (Cohn) Schroet.	–	–	–	–	–	–	×	–	–	–
Helicostylum pyriforme Bain.	–	×	×	–	–	–	–	×	×	–
Aspergillus niger van Tiegh.	×	×	×	×	×	×	×	×	×	×
A. nidulans (Eid.) Wint.	×	×	×	×	×	×	×	×	×	×
A. terreus Thom	×	×	×	×	×	–	×	×	×	×
A. melleus Yukawa	×	×	×	–	–	–	×	×	×	×
A. sydowi (Bain. & Sart.) Thom & Church	×	×	×	–	–	–	×	×	×	×
A. flavus Link ex Fr.	×	×	×	×	–	–	×	×	×	×
A. versicolor (Vuill.) Tirab.	×	×	×	–	–	–	×	×	×	×
A. flavipes Bain. & Sart.	×	×	×	–	–	–	–	×	×	×
A. tamarii Kita	×	–	×	–	–	×	–	–	–	×
A. nidulans (Eid.) Wint. var.nov.	–	–	×	–	–	–	–	–	–	–
A. candidus Link ex Fr.	×	×	×	×	–	×	×	×	×	×
A. fumigatus Fresen. var.nov.	×	×	–	–	–	–	×	–	–	×
Penicillium funiculosum Thom	×	×	×	–	–	–	×	×	×	–
P. corylophilum Dierckx	×	×	×	–	–	–	–	–	–	–
Paecilomyces varioti Bain.	–	–	×	–	–	–	×	×	×	×
Gliocladium roseum Bain.	–	–	–	–	–	–	×	–	–	×
Alternaria tenuis Nees	×	×	×	×	×	–	×	×	×	×
Curvularia lunata (Wakker) Boedijn	×	×	×	×	×	×	×	×	×	×
Cladosporium cladosporioides (Fres.) De Vries	×	×	×	–	×	×	×	×	×	×
C. sphaerospermum Penzig	×	×	×	×	×	×	×	×	×	×
Trichothecium sp.	×	×	×	–	–	–	×	–	–	–
Thielavia sp.	–	–	–	–	–	–	×	–	–	–
Fusarium solani sensu Snyder & Hansen	×	×	×	–	–	–	×	×	×	×
F. scirpi Lamb. & Fautr. var. *compactum* Wr.	×	×	×	–	–	–	×	–	–	–
Corticium solani (Prill. & Delacr.) Bourd. & Galz.	×	×	×	–	–	–	–	×	×	×
Total number of species	26	28	29	9	8	8	26	21	22	22

CONCLUSION

The fungi obtained by the different methods are similar in type and in degree of prevalence to those recorded from similar soils in other warm countries (Sabet 1935; Chaudhuri & Sachar, 1934; Todd, 1932; Jensen, 1931; Waksman, 1917; Werkenthin, 1916). Although climate and soil are closely interdependent, it appears that climatic more than edaphic similarities have resulted in this similarity of soil fungal floras.

I am grateful to the Director and staff of the Commonwealth Mycological Institute for their help in the identification of the fungi. Voucher

cultures are available in the mycological herbaria of the C.M.I. and the Faculty of Agriculture, Khartoum North. I am also grateful to Mr G. A. Worral for assistance in the soil analyses.

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CONIDIA OF *ACROSPERMUM COMPRESSUM* AND *A. GRAMINUM*

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(With 2 Text-figures)

Acrospermum compressum and *A. graminum*, which have earlier been considered as synonyms, produce different types of conidial apparatus in culture. *A. compressum* forms two-celled conidia, whilst *A. graminum* forms unicellular conidia.

Acrospermum compressum Tode ex Fr. and *A. graminum* Lib. show characteristic differences when grown in culture. The two species have, however, been regarded as one. Rehm (1896), Riddle (1920) and Brandriff (1936) regarded *A. graminum* as a variety of *A. compressum*. This view is based on the similarity of fruit-bodies of the two species, but no cultural evidence was put forward to support it, although Brandriff grew *A. compressum*. She states that 'after three weeks fruiting structures developed. These had the appearance of spermogonia or very small pycnidia. They were provided with short beaks, and contained minute unicellular bodies of the aspect of spermatia.' No drawings or measurements of these structures were given. Cultures from recent British collections differ from those described by Brandriff.

Acrospermum compressum

Isolations of *A. compressum* have been made from the following five collections:

Herb. University Sheffield number	Host	Locality	Date
1178	*Urtica dioica*	Howldale, Pickering, Yorks. Coll. W. G. Bramley	13. vi. 54
1643*a*	*U. dioica*	Middle marsh, Wheatfen Broad, Surlingham, Norfolk	12. iv. 55
1647	*U. dioica*	Lowdales, Hackness, Yorks	16. iv. 55
1648	*Galium aparine*	Lowdales, Hackness, Yorks	16. iv. 55
1649	*Urtica dioica*	Forge Valley, Yorks.	15. iv. 55

Isolations from all these collections produced a similar type of growth in culture and the description below is based on specimen no. 1643*a*.

Fruit-bodies (Fig. 1A): single or in groups, club-shaped to cylindrical, slightly compressed, pointed, pale yellow to brown and somewhat translucent when moist, darker on drying, usually 2–3 mm. high and 200–400 μ broad. The wall of the fruit-body is about 50–60 μ thick, composed of parallel narrow hyphae, turning outwards and expanding at their tips to form a pseudoparenchymatous outer layer of brown polygonal cells

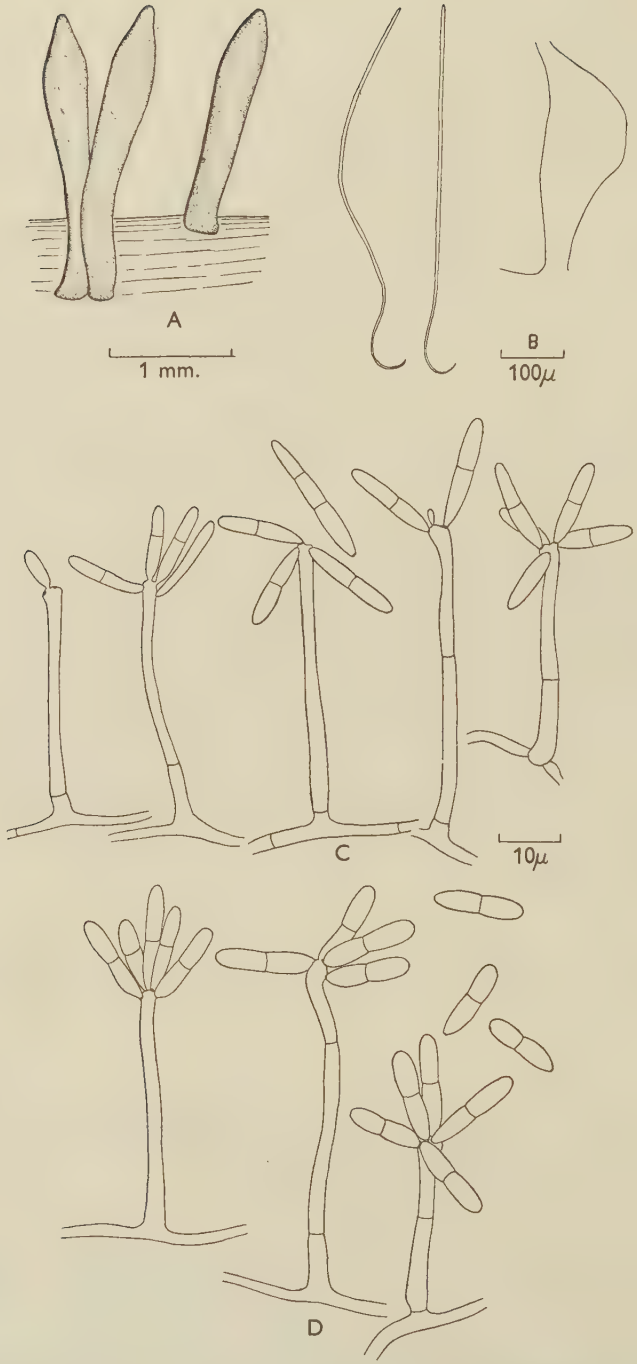

Fig. 1. *Acrospermum compressum*. A, B, C from Herb. University Sheffield, no. 1643*a*; D from 1178. A, fruit-bodies on *Urtica* stem; B, asci and ascospores; C, conidia from a single ascospore culture, 14 days old on maize-extract agar; D, conidia from a similar culture derived from Herb. no. 1178.

about 10 μ wide. *Asci* (Fig. 1 B): confined to the wider upper part of the fruit-body, fasciculate, narrowly cylindrical, up to 500 μ long, 3–4 μ wide, separated by numerous filiform paraphyses. *Ascospores*: 8, filiform, hyaline, almost as long as the asci, about 0·5 μ wide.

This specimen has been matched with Schmidt and Kunze Exsiccatum no. 69, also on *Urtica dioica*, which was one of the exsiccati cited by Fries (1822).

Cultures. From a suspension of crushed asci, single ascospore and single ascus cultures were prepared. On maize-extract agar the fungus grew slowly, forming flat whitish colonies. The hyphae were at first confined to the medium, 1–3 μ wide, often forming characteristic coiled skeins. Later aerial hyphae were produced, and these bore conidiophores within 10–14 days of inoculation. The conidiophores were mostly unbranched and consisted of pale yellow upright hyphae 20–80 μ long, 3–4 μ wide at the base, tapering to 2 μ near the apex. The tip of the conidiophore bore a radiate group of cylindrical conidia, and up to fifteen conidia have been counted on a single head (Fig. 1 C, D). The first conidium arises as the blown-out tip of the conidiophore. Subsequent conidia are formed close to it (probably acrogenously) so that a close crown of conidia arises. When a conidium is detached an inconspicuous flattened scar is visible at the conidiophore apex. Mature conidia are pale yellow in mass, cylindrical to club-shaped, tapering to a flattened scar below, rounded above, usually with a single median transverse septum, slightly constricted. Occasional 2- to 3-septate spores were found, and in old cultures the individual cells of the spores were often inflated. The ranges in size of the conidia from the five collections were: 1178, 10–20 × 3–4 μ; 1643*a*, 13–23 × 2·5–3 μ; 1647, 12–30 × 3–4 μ; 1648, 12–18 × 2·5–3 μ; 1649, 8–18 × 3–4 μ.

Acrospermum graminum

Isolations of *Acrospermum graminum* have been made from the following four collections:

Herb. University Sheffield number	Host	Locality	Date
365	Wheat stems	Bolton Percy, Yorks. Coll. W. G. Bramley	1. v. 50
1055	*Deschampsia caespitosa*	Fulwood, near Sheffield	5. vi. 51
799	*Agropyron repens*	Kilnsea, E. Yorks	21. vii. 49
1644	*Phragmites communis*	Home Marsh, Wheatfen Broad, Surlingham, Norfolk	12. iv. 55

All isolates produced similar growth in culture. The following description is based on specimen no. 365.

Fruit-bodies (Fig. 2 A): club-shaped, 1–1·2 mm. high and 200–300 μ wide, yellow to brown in colour, single or in groups of two to four, seated on a broader base and bursting through the epidermis of the leaf sheath, rounded or pointed at the apex, slightly flattened above, collapsing on drying to form shallow furrows. The wall of the fruit-body is made up of narrow parallel, interwoven hyphae, turning outwards to form rounded or polygonal yellowish brown cells about 6 μ wide. *Asci*: fasciculate,

narrowly cylindrical, tapering at the base and rounded above, 400–600 μ long, 4–6 μ wide, separated by filiform paraphyses. *Ascospores*: 8, filiform, hyaline, about as long as the asci, 0·5 μ wide.

The specimen has been matched with Libert's Pl. Crypt. Arduen. no. 33.

Cultures. Six cultures were prepared from ascospores and asci. The asci and ascospores germinated readily and were transferred to a variety

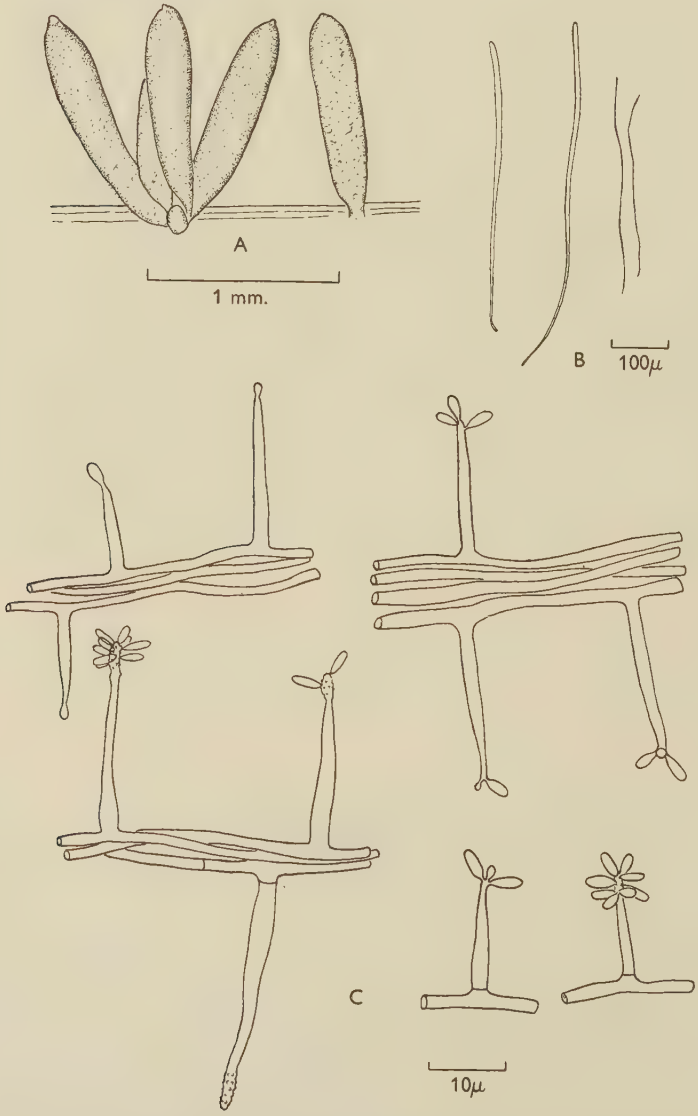

Fig. 2. *Acrospermum graminum*. Herb. University Sheffield no. 365. A, fruit-bodies on wheat stems; B, asci and ascospores; C, conidia from single ascospore culture, 14 days old on maize-extract agar.

of agar media. The fungus grew rather slowly, producing regular circular colonies with a submerged cream-coloured mycelium, and an aerial growth which was faintly green in colour, forming rope-like strands. Within 10 days conidia were produced on short conidiophores projecting from the rope-like strands of aerial mycelium or from the submerged mycelium. Conidia were produced in all cultures, including one derived from a single ascospore and one from a single ascus. The conidiophores are tapering and unbranched and occasionally bear a septum at the base, are 15–16 μ long × 2·5–4 μ wide at the base tapering to 1·5–2 μ at the apex. The first formed conidium is produced terminally as a cylindrical swelling at the tip of the conidiophore and is displaced laterally by the formation of a second growing point, which grows beyond the first formed conidium and produces a second conidium. This process is repeated so that an 'ear' of conidia is formed. Eventually in culture, hundreds of conidia may be formed in a single head. When conidia are displaced a series of small peg-like scars can be observed on the conidiophore (Fig. 2C). The conidia are hyaline, cylindrical or elliptical, and measure 3–6 × 1·5–2·5 μ (mostly 4 × 1·5 μ). Similar conidiophores and conidia have also been found developing from the wall of the fruit-body, after incubation in a damp-chamber.

No appreciable difference has been detected in the growth and dimensions of the other isolates. In addition to these isolates, the fungus has been isolated repeatedly from the washed fragments of upper internodes of standing stems of *Agropyron repens*, collected at Lindrick Common, near Worksop by Mr H. J. Hudson and from fragments of standing stems of *Dactylis glomerata*.

Zogg (1950) records isolating *Acrospermum compressum* var. *graminum* from cereals, but does not describe his isolates.

Discussion

Comparison of over fifty collections of *Acrospermum* on herbaceous stems and grasses from Herb. Kew. showed great variation in size and shape. However, the height of fruit-bodies in collections on grasses rarely exceeds 1·5 mm., whilst on herbaceous stems they are frequently larger, often up to 3 mm. The similarity of fruit-bodies on the two types of host has doubtless led earlier investigators to regard them as representing a single species. However, the differences in conidial apparatus suggest that they should be regarded as separate species. It would be of interest to study isolates of *Acrospermum* on hosts such as *Typha* and *Carex*, on which it is known to occur, to see which species is present. *A. foliicolum* Berk., which grows on leaves of deciduous trees in the Southern states of North America, resembles *A. compressum*, and Riddle (1920) has regarded it as a variety of the latter. It would be of interest to study isolates of this fungus.

The discrepancy between Brandriff's description of conidia of *A. compressum* and the present isolates is puzzling. In none of the British isolates has pycnidia been observed, although the fungus has been kept in culture for several months. I have examined Brandriff's collections and slides. Her collections on *Caltha* are more robust than those on grasses and sedges,

and it seems to me probable that the former should be ascribed to *A. compressum* and the latter to *A. graminum*.

The taxonomic position of *Acrospermum* has puzzled most authorities. Rehm (1896) has reviewed earlier dispositions of the genus. Saccardo (1883) classified it with the Hysteriaceae-Scolecosporae, dividing the group into two series: (A) Genuinae and (B) Desciscentes (lit. degenerate, spurious), placing *Acrospermum* in the latter group. Rehm himself erected the group Pseudohysterineae for the genus. Brandriff (1936) also indicated relationship to the Hysteriales. Luttrell (1955) places the genus in the Coryneliales, but suggests that it should possibly be considered 'as a partially reduced perithecial form and placed in the vicinity of the Clavicipitaceae'. However, the asci of *Acrospermum* do not possess the characteristic apical apparatus found in *Claviceps*, and a satisfactory disposition of the genus is still awaited.

It is a pleasure to thank Mr W. G. Bramley and Mr H. J. Hudson for specimens. I am also grateful to Dr R. P. Korf, Curator of the Herbarium, Department of Plant Pathology, Cornell University, for allowing me to examine Miss Brandriff's collections, and to the Directors of the Herbaria of the Royal Botanic Gardens, and the Commonwealth Mycological Institute at Kew, for permission to study specimens and literature. I am further indebted to the University of Sheffield Research Fund for a grant in aid of this study.

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INFECTION OF STRAWBERRY ROOTS BY ZOOSPORES OF *PHYTOPHTHORA FRAGARIAE*

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(With Plate 13 and 4 Text-figures)

The period of motility of zoospores of *Phytophthora fragariae*, produced in non-sterile pond water, was longest, up to approximately 5 hr., at 13–14° C. and at pH values of 6·0 and 6·8. Sterilization of the pond water by heat or by seitz-filtration retarded and depressed sporangial production. In seitz-filtered water, and in distilled water (in which sporangia were not formed at all), there was profuse mycelial development.

Zoospores of *P. fragariae* encysted in large numbers on the roots of immune, resistant and susceptible strawberry cultivars and on the roots of non-host plants. They encysted just as readily on roots killed by immersion in boiling water but did not encyst on fine glass rods or on strands of wool. Zoospores encysted in the root-hair zone and at the root-tips. The germ-tubes of cysts germinating in the root-hair zone did not penetrate the root but grew away from it in all directions. At the root-tip, germination was followed by penetration both in strawberries susceptible and immune to infection and in non-host plants. Penetration did not extend beyond the epidermal layer except in susceptible strawberry cultivars in which, within 3 days, the fungus reached the differentiating stele where it was thereafter confined, growing within the phloem and pericycle.

Introduction

From field observations at Westerham Hill, Kent, on Red Core disease of strawberries caused by *Phytophthora fragariae*, Hickman (1940) concluded that the disease was most severe under waterlogged conditions. He found sporangia growing out from the tips of infected roots and suggested that within a field the pathogen was distributed by transport of its zoospores in soil drainage water. Some outbreaks of Red Core, where the drainage was free, were thought to be due to transport of zoospores in drainage water from neighbouring contaminated waterlogged areas. Later (Hickman, 1946; unpublished), in the Tamar Valley, Cornwall, he found Red Core spreading rapidly in strawberries growing on steep slopes where drainage was not impeded. This apparent contradiction was explained by Hickman & English (1951), who showed experimentally that most infection occurs under high soil moisture conditions which favour the liberation of zoospores and their carriage through the soil. Such conditions are present both in areas where rainfall is low to moderate, and drainage poor, e.g. at Westerham Hill, and in well-drained areas such as the Tamar Valley, where rainfall is high. From their observations it was clear, therefore, that zoospores played an extremely important part in the spread of the

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disease, and it was with this fact in mind that the present study was made of the behaviour of zoospores in relation to strawberry roots, both of cultivars susceptible and resistant to Red Core. In addition, the observations of Hickman (1940) on the process of invasion of the root tissues by the fungus were extended.

Factors affecting asexual reproduction

As a preliminary to encystment experiments some observations were made on the influence of temperature and of pH on the period of motility of zoospores. The only published observations on zoospore activity of *P. fragariae* are those of Bain & Demaree (1945), who observed that at 10–15° C. zoospores were motile for $4\frac{1}{4}$ hr., there being a conspicuous reduction in numbers and activity of swimming zoospores during the last three-quarters of an hour. Without giving further details, they stated that zoospores are exceedingly sensitive to temperature, as well as to the composition of the liquid into which they are released.

Table 1. *Effect of temperature on behaviour of zoospores of* Phytophthora fragariae

Time (hr.)	5–6° C.	13–14° C.	20° C.	26° C.	34° C.	44° C.
$\frac{1}{4}$	Z+ C+++	Z+++ C+	Z+++ C+	Z+ C+++	Z+ C+++	Z− C+++
$\frac{1}{2}$	Z+ C+++	Z++ C++	Z+ C+++	Z− C+++	Z− C+++	Z− C+++
$1\frac{1}{2}$	Z+ C+++	Z++ C+++	Z− C+++	Cysts germinating	Cysts disintegrating	Cysts disintegrating
5	Z− C+++	Z+ C+++	Germ-tubes 13·5–108 μ	Germ-tubes 27–108 μ	—	—
24	Germ-tubes *c.* 13·5–40·5 μ	Z− Germ-tubes 40·5–202 μ	Germ-tubes 67–270 μ	—	—	—

Key. Z−, no zoospores motile; Z+, few zoospores motile; Z+++, many zoospores motile; C−, no cysts; C+, few cysts; C+++, many cysts.

Using the method for zoospore production described by Hickman & English (1951), i.e. incubating disks of bean-agar cultures in non-sterile pond water at 13° C., a standard volume of zoospore suspension was placed in a number of Petri dishes which were distributed, in triplicate, at six different temperatures over the range 5–44° C. Observations on zoospore activity, encystment and germination during the following 24 hr. are summarized in Table 1.

The optimum temperature for zoospore activity was 13–14° C., some swimming zoospores being observed after 5 hr. A few motile zoospores were seen after $1\frac{1}{2}$ hr. at 5–6° C., but at this temperature most of the zoospores had encysted after 15 min. exposure. At 20° C. encystment was complete after $1\frac{1}{2}$ hr., and at temperatures above 20° C. activity ceased after 15–30 min. exposure.

The effect of pH on zoospore activity was examined at 13–14° C. in mixtures of equal parts of zoospore suspension and buffer solutions

(Table 2). For each pH level two Petri dishes were filled with 20 ml. of the appropriate solution to which was added 20 ml. zoospore suspension.

The optimum pH values for zoospore activity were 6·0 and 6·8. If the solution was more acid or more alkaline, the zoospores were killed rapidly, often dying without encysting. The difference between the duration of activity in the mixture of pond water and buffer solution and in the pond water alone, both at pH 6·8, was thought to be due to the mechanical disturbance which occurred in the former when the zoospore suspension was added to the solution. In many other experiments it was noticed that any form of mechanical disturbance of the zoospore suspension, such as stirring or shaking, led to more rapid encystment.

Table 2. *Effect of* pH *on activity of zoospores of* Phytophthora fragariae

Composition of buffer mixture			Duration of activity of zoospores	
0·1 M citric acid in ml.	0·2 M disodium phosphate in ml.	pH	hr.	min.
11·18	8·82	4·4	–	5
9·28	10·72	5·2	–	45
7·37	12·63	6·0	2	30
4·55	15·45	6·8	2	35
1·83	18·17	7·4	–	30
0·55	19·45	8·0	–	30
Pond water alone		6·8	3	40

It was also observed, as was expected, that the source of pond water influenced the number of sporangia and zoospores produced. In the following experiment, samples of pond water were obtained from six different sources in the Birmingham area. A portion of each sample was passed through a seitz-filter, another sterilized in the autoclave at 15 lb. pressure for 20 min., and a third left unsterilized. Ten small bean-agar disks were placed in each of a number of Petri dishes containing the different waters. Observations made on sporangium production and zoospore activity after 24, 48 and 60 hr., at 13–14° C., are summarized in Table 3.

These results confirm and extend unpublished observations by Hickman, and by Fleetwood-Walker on the influence of sterilization on asexual reproduction of *P. fragariae* in pond water. With one exception, D, sporangia and zoospores were present in abundance in non-sterile pond water after 48 hr. This was also true for the autoclaved pond water from two sources, namely B and E, but in the others heat sterilization had depressed asexual reproduction. Sterilization by seitz-filtration had an even more depressing effect on sporangium production. Few were produced and these only after a long period. Associated with this poor development of sporangia was great vegetative growth. The hyphae were very branched and formed an extremely dense fringe round the disk. It was interesting to find that the behaviour of the fungus was very similar in distilled water whether sterilized or not. Here, however, no sporangia were produced whatsoever.

Table 3. *Effect of different water supplies from the Birmingham area on development of mycelium, sporangia and zoospores of* Phytophthora fragariae

Source of water	Autoclaved				Seitz-filtered				Non-sterile			
	pH	24 hr.	48 hr.	60 hr.	pH	24 hr.	48 hr.	60 hr.	pH	24 hr.	48 hr.	60 hr.
A. Lickey hills												
M	6·9	4+	4+	4+	7·0	6+	6+	6+	7·0	4+	4+	4+
S		2+	2+	4+		–	–	2+		4+	4+	4+
Z		–	2+	2+	–	–		2+		4+	4+	4+
B. Manor house												
M	6·6	4+	4+	4+	6·0	6+	6+	6+	6·8	4+	4+	4+
S		4+	4+	4+		–	–	2+		4+	4+	4+
Z		4+	4+	4+		–	–	2+		4+	4+	4+
C. Winterbourne												
M	7·0	4+	4+	4+	6·9	6+	6+	6+	6·7	4+	4+	4+
S		–	2+	2+		–	–	2+		2+	4+	4+
Z		–	–	2+		–	–	2+		–	4+	4+
D. Harborne reservoir												
M	6·2	4+	4+	4+	6·7	6+	6+	6+	6·2	4+	4+	4+
S		–	2+	2+		–	–	–		2+	2+	2+
Z		–	–	2+		–	–	–		–	2+	2+
E. Swanshurst lake												
M	6·9	4+	4+	4+	7·2	6+	6+	6+	7·1	4+	4+	4+
S		2+	4+	4+		–	–	2+		4+	4+	4+
Z		–	4+	4+		–	–	2+		2+	4+	4+
F. Distilled water												
M	4·9	6+	6+	6+	6·8	6+	6+	6+	4·7	6+	6+	6+
S		–	–	–		–	–	–		–	–	–
Z		–	–	–		–	–	–		–	–	–

Key. M, mycelium; S, sporangia; Z, zoospores; –, none; 2+, few; 4+, abundant; 6+, very abundant.

Encystment experiments

Strawberry roots were inoculated by immersing the root systems of runner plants in zoospore suspensions prepared at 13–14° C. in non-sterile pond water of pH 6·8, following the technique described by Hickman & Goode (1953). Large (5–6 cm.) colonies of *P. fragariae* growing on bean agar, were immersed in non-sterile pond water. After 24–48 hr. at 13–14° C. a dense fringe of sporangia was present at the edges of the disks (Pl. 13, fig. 1), which liberated zoospores in large numbers. Encystment occurred some 3–6 hr. after immersing the roots, and these were then removed for staining to lactophenol containing 0·01 % cotton blue. After a few hours excess stain was removed in pure lactophenol, when the cysts were clearly recognizable. To make comparative records of encystment, five counts were made at measured intervals over the whole length of each root, under low-power magnification, each count including cysts on either side of the piece of root, at one depth of focus. Using this method, approximately one-quarter of an inch of each root was recorded.

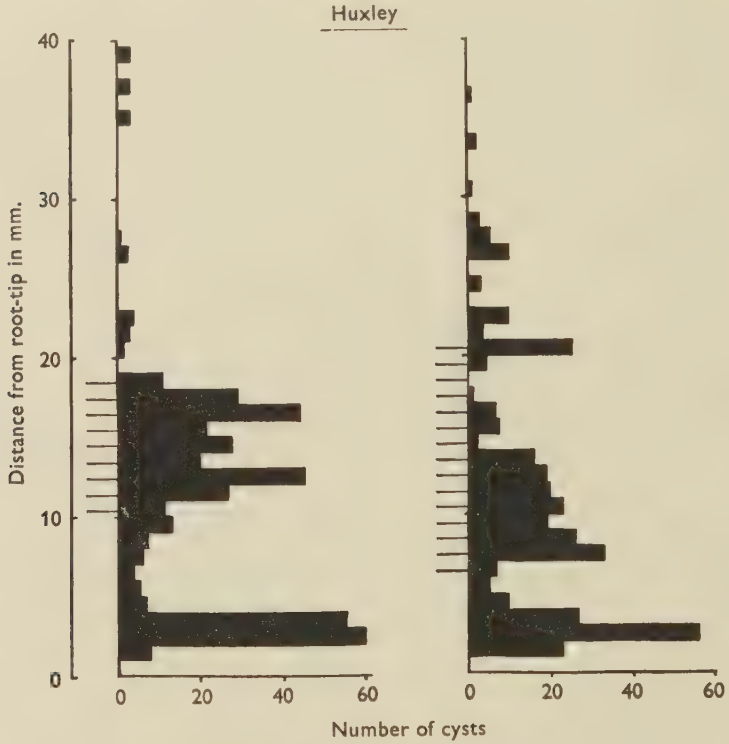
Since differences in susceptibility to infection by *P. fragariae* exist among strawberry cultivars it was natural to compare first of all, the encystment of zoospores on the roots of susceptible and immune cultivars. The root systems of three Huxley and three Climax runner plants were immersed for 24 hr. in a zoospore suspension of a race of *P. fragariae* pathogenic to Huxley but not to Climax. Two roots from each of three plants were then removed, and counts made of the encysted zoospores using the technique described. A total of 224 cysts were counted on the Huxley roots and 233 on the Climax roots. It was clear that zoospores encyst equally well on both Huxley and Climax roots. This type of experiment was repeated twice, first using the same two varieties, with an isolate which was highly pathogenic to both of them; and secondly, using Huxley and Perle de Prague, with an isolate which was highly pathogenic to Huxley and less pathogenic to Perle de Prague. In none of these experiments were any significant differences observed in the numbers of zoospores encysting on the roots of the two varieties.

Similar experiments with other plants (*Geum rivale*, *Potentilla atrosanguinea*, *Senecio vulgaris* and *Ranunculus repens*), in comparison with strawberry, demonstrated that zoospores of *P. fragariae* encyst just as freely on the roots of non-host plants as they do on strawberry roots. It was impossible to make precise comparisons between the different plant species in respect of number of zoospores encysting, as the root systems varied so considerably. However, it was observed that large numbers of zoospores encysted in the root-hair region in all plants, and the phenomenal numbers of cysts found on the roots of *R. repens* were associated with great root-hair development in that species.

In addition, further experiments showed that roots which had been killed by immersion for 10–15 min. in boiling water provided just as good a substrate for encystment as living roots, but very few zoospores encysted on fine glass rods or on strands of wool.

Mention has already been made of differences in the concentration of encysted zoospores along roots. It was observed consistently that there

were many cysts at the tips of roots, and they were also present in large numbers in the root-hair zone. Precise counts along the entire length of roots of the cultivars Huxley and Climax, which had been immersed in zoospore suspension for 24 hr., confirmed these observations. The distribution pattern is shown in the form of a histogram in Text-fig. 1.



Text-fig. 1. Distribution pattern of cysts of *Phytophthora fragariae* on roots of strawberry cultivar Huxley.

Cysts were never observed at the very apex of roots, for a distance of about 1 mm. from the tip, but occurred in large numbers in an area extending for 2–3 mm. immediately behind this zone, and in the root-hair zone (indicated on the left of the histogram by fine horizontal lines). Over the rest of the root, cysts were present only in very small numbers. Observations were also made on roots with few or no hairs, and here the greatest number of cysts occurred at the root-tip.

The path of infection

Following this study of zoospore encystment, observations were made on the germination of the cysts and on the subsequent development of the parasite, with the strawberry cultivars Huxley and Climax (susceptible and immune respectively, to the race of *P. fragariae* used), *Ranunculus* and *Geum*.

Whole mount observations after 24 and 48 hr., on roots immersed in zoospore suspensions, indicated that the cysts at the root-tip had germinated earlier than those in the root-hair zone where germination was at random, most of the germ-tubes growing away from the root. After this period the inoculated plants were potted up in sand and 4 days later the roots of the susceptible Huxley showed the typical vascular discoloration behind a purplish brown area at the tips.

More detailed observations on the roots of Huxley and Climax removed from the zoospore suspension at different time-intervals showed that germination of cysts at the root-tip had occurred within 3 hr. of placing the roots in the zoospore suspension and germ-tubes had penetrated the fragmentary root-cap layer in both cultivars. With the aid of stains (cotton blue, acetocarmine) a few hyphae could be seen within the cortex of the Huxley roots after 22 hr., whereas in Climax no further development had apparently taken place, the cysts remaining with small germ-tubes.

To obtain more detailed information, infected root material of the four plants was embedded in paraffin at successive time-intervals and sectioned by microtome. The plants were placed for 24 hr. with their root systems in a zoospore suspension and then transferred to water.

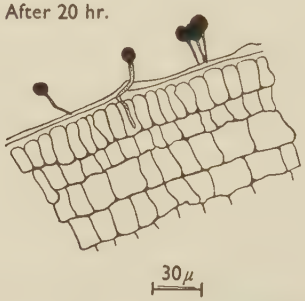
Formalin–acetic–alcohol (100 ml. 50 % alcohol, 6·5 ml. formalin, 2·5 ml. acetic acid) was the most satisfactory fixative and the chloroform–alcohol method of infiltration as described by Johannsen (1940) was employed. Sections were cut at 10–15 μ and several methods of staining were tried. The carbol-thionin and orange-G technique described by Stoughton (1930) proved quite satisfactory for the more mature root material but had to be modified for root-tips. If the sections were left in carbol-thionin for 1 hr., differentiation was almost impossible because the meristematic cells became very deeply stained. The best results were obtained by leaving the sections in carbol-thionin for 20–30 min. and differentiating in orange G for 1–3 min. Of the other methods used, the periodic acid-Schiff technique described by Dring (1955) proved highly satisfactory.

The first stage in the infection of Huxley roots by a highly pathogenic isolate of *Phytophthora fragariae* is illustrated in Text-fig. 2.

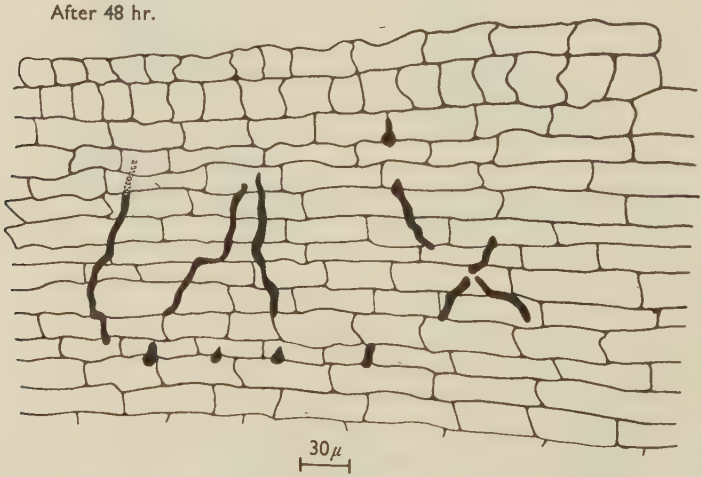
After approximately 20 hr., many of the cysts in the root-tip zone had produced germ-tubes, and as they still contained protoplasm the stain was taken up readily. The germ-tubes appeared granular, and were fine, thread-like structures approximately 18·0–50 μ in length. The longer germ-tubes had penetrated the fragmentary remains of the root-cap layer, but the shorter ones seemed only slightly attached to it. In the illustration, the cysts appear to have been pushed away from the root, sometimes for some distance. This may have been a result of the fixing and staining procedures. In all the preparations there was this rather fragmentary outermost layer of cells which comprise the root-cap tissue. In general the root-cap extends back for 1–1·5 mm. and the illustration represents a portion of tissue almost at the limit of the root-cap zone.

A few empty cysts could be seen, but the course of the germ-tubes which had penetrated the root-tip could not readily be traced, due to the large amount of stain taken up by the cells of this meristematic region. Germ-tubes were also seen penetrating directly into the epidermal layer

at distances up to 4 mm. behind the root-cap. Here the cells were larger and penetration could more readily be observed. Beyond this area, the number of cysts was relatively very small; some of them had not germinated and very few hyphae could be observed in the cortex. No penetration whatsoever was observed beyond a zone 6–7 mm. from the root-cap. The germ-tubes did not form appressoria and no distortion of root tissue was



Text-fig. 2. First stage in penetration of roots of susceptible, Huxley cultivar by *Phytophthora fragariae*. The irregularly parallel lines outside the epidermis represent the appearance of sloughing-off root-cap tissue.



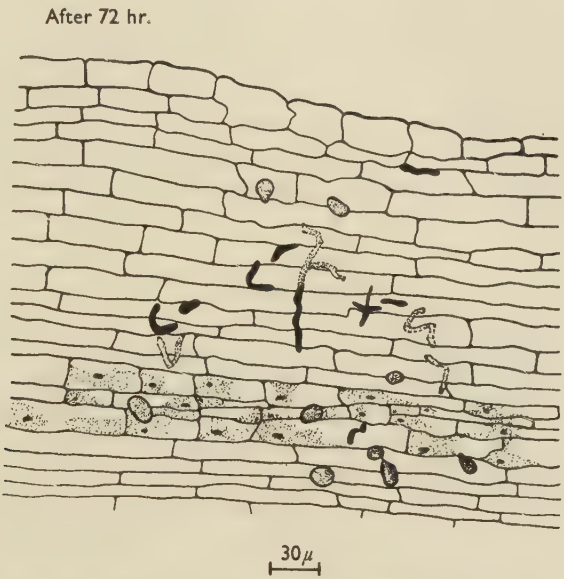
Text-fig. 3. Illustrating extent of invasion in root of susceptible, Huxley cultivar by *Phytophthora fragariae* after 48 hr.

associated with their entry. They penetrated both between and directly through the epidermal cells, but only the latter type of penetration is illustrated in Text-fig. 2.

Text-fig. 3 represents a section through the root, just beyond the limit of the root-cap area, after 48 hr. No cysts were visible on the epidermal layer but fragments of mycelium 10–95 μ in length could clearly be seen both within and between the cortical cells. The portions of hyphae which were advancing through the cells of the cortex were deeply stained, whereas many of the older portions were granular in appearance and often

difficult to distinguish clearly. It seemed that the protoplasm was constantly advancing with hyphal growth, leaving emptied portions in infected cells. The hyphae appeared to penetrate the walls of the host cells quite readily, without causing any distortion. Although there was a certain amount of branching of the hyphae in the cortex, the general advance was in the direction of the stele, which was, in this region, in the early stages of differentiation. Some small fragments of hyphae were observed passing through the procambial strands into the undifferentiated stelar core.

After 72 hr. hyphae could be seen throughout the cortical tissue behind the root-cap and in the differentiating stele (Text-fig. 4).



Text-fig. 4. Illustrating development of *Phytophthora fragariae* in root of susceptible, Huxley cultivar after 72 hr. The stippled, elongated cells in the lower half of the figure represent procambial strands.

They again appeared very fragmentary and some terminal portions contained protoplasm while others were empty. The early stages of oospore development were observed at this time in the cortical cells and in the differentiating stelar zone. These reproductive bodies appeared as small, rounded, granular masses. Occasionally, adjoining hyphae were recognizable, but more often they were empty, not easily visible and the sex organs seemed isolated structures, lying within or between the cells.

In the later stages of infection, e.g. after 96 hr., many empty hyphae were observed in the cells of the cortex. On close examination, the hyphae could often be seen to have forced a passage between the walls of adjoining cortical cells, as well as passing directly through them, and many small gaps were present, marking points of such intercellular passage. Hyphal strands had passed back to the differentiated stelar tissue where, together with oogonia and antheridia, they were prominent in the phloem and

associated parenchyma. At this distance (2–3 mm.) from the root-tip, sex organs were far more common in the stele than in the cortex.

As the fungus continued to colonize the root and grew toward the stock, away from the zone of penetration, it became confined to the stele. Within the stele it was present mainly in the phloem and pericycle. At this stage, the mycelium was not easily ditinguishable, because it consisted largely of empty hyphae. The largest number of reproductive organs were found in the phloem (Pl. 13, fig. 2) and often they had caused distortion by the crushing of phloem and pericycle cells. Occasionally, they were observed within the pericycle itself but never in the xylem.

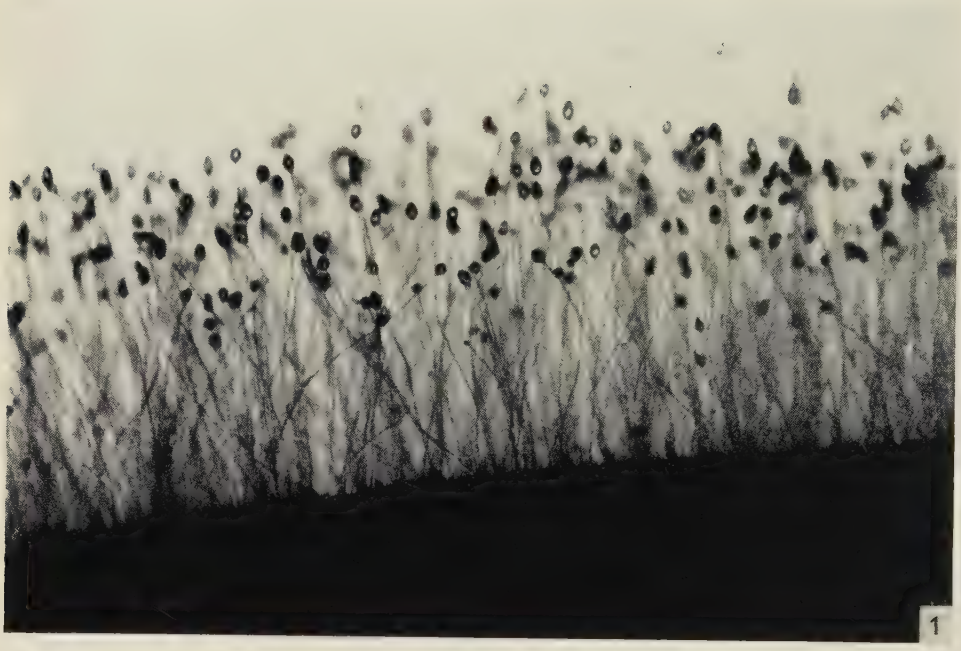
While this particular isolate of *P. fragariae* readily infected the roots of the strawberry cultivar Huxley, it did not do so in Climax, nor in *Ranunculus* and *Geum*. After these plants had been immersed in a suspension of zoospores for 24 hr., cysts could be seen on the cells of the root-cap, and the germ-tubes appeared to penetrate this fragmentary layer as in Huxley, but no further growth occurred. Root material examined after 48 and 72 hr. showed no mycelium in the cortical cells. It appeared, therefore, that the germ-tubes never actually penetrated beyond the epidermal layer.

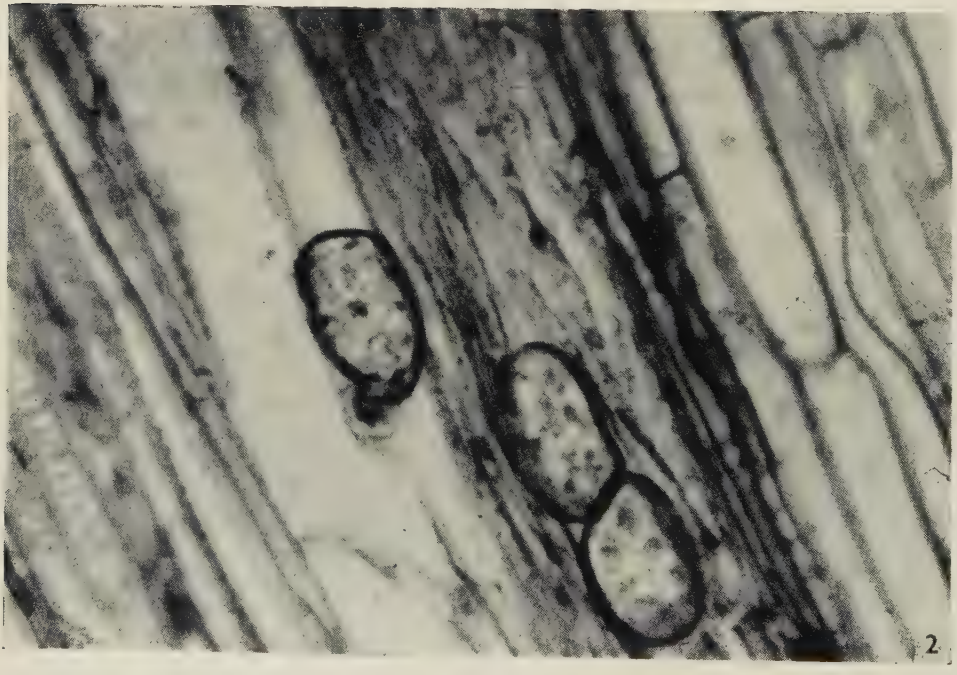
Discussion

In all the encystment experiments, whether with strawberry or with the non-host plants *Ranunculus*, *Geum* and *Senecio*, there was the same definite pattern of encystment. Zoospores encysted in large numbers in the root-hair and root-tip regions. Very few encysted elsewhere. In the root-hair zone it seemed that encystment was probably a result of trapping of zoospores, still motile but possibly moving sluggishly, late in their motile stage. Although the cysts germinated in this zone the course of the germ-tubes was completely undirectional. They were often found growing away from the roots and penetration of root-hairs, or of the root itself in this zone, was never observed.

In contrast, encystment in the root-tip zone appeared to be a response to a stimulus which first attracted the zoospores and then induced penetration by their germ-tubes. When roots were immersed in a zoospore suspension encystment and germination always occurred first in the root-tip zone. Moreover, germination was not at random here, for the germ-tubes penetrated directly into the root tissue. Zoospores displayed the same behaviour in relation to roots of the non-host plants used, suggesting that the stimulus is identical, or very similar, in all these roots. The nature of the stimulus has not been investigated.

After penetration of the root-tips of strawberry cultivars susceptible to infection by *P. fragariae*, the hyphae grow directly towards the differentiating stele within which they are confined to phloem and pericycle. In an immune variety and in unrelated plants, penetration does not extend beyond the outer layer of root-tip cells. No anatomical differences in root-tip structure which would explain differences in susceptibility could be demonstrated. It is concluded, therefore, that resistance to *P. fragariae* is physiological in nature.





(*Facing p.* 377)

I am indebted to Dr C. J. Hickman for advice during the course of this work, which was completed while I was a member of the Agricultural Research Council Unit of Biometrical Genetics, University of Birmingham.

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EXPLANATION OF PLATE 13

Fig. 1. Sporangia of *Phytophthora fragariae* growing from edge of bean-agar disk in non-sterile pond water. (×40.)

Fig. 2. Oogonia and antheridia of *Phytophthora fragariae* in the phloem of an infected strawberry root. (×400.)

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THE SPORE DEPOSIT OF *DALDINIA*

By C. T. INGOLD

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(With Plate 14)

If a thick median slice is cut from an active stroma of *Daldinia concentrica* and laid horizontally on a glass plate, a spore deposit collects overnight as a black band about 1·0 cm. wide and separated from the edge of the stroma by a more or less spore-free region 0·2–0·3 cm. wide. Further the spore deposit is zoned with several darker regions parallel with the surface of the stroma alternating with paler zones. It is suggested that both the considerable width of the deposit and its zoning are associated with the discharge of projectiles ranging from single spores to groups of eight.

If an active stroma of *Daldinia concentrica*, which is approximately hemispherical, is placed on a sheet of glass, covered to eliminate draughts and left overnight, there accumulates around it a spore deposit in the form of a black band extending outwards to a distance of about 1·2 cm. from the edge of the stroma. The only information that this gives is that the maximum distance of discharge is about 1·2 cm. In some recent experiments spore deposits were obtained not from whole stromata but from semicircular median vertical slices 0·5–0·7 cm. thick. Each slice was placed flat on a clean horizontal glass plate and left overnight. A typical spore-deposit obtained in this way is shown in Pl. 14*a*. It will be seen first that the deposit does not take the form of a line parallel with the surface, but is a broad band 0·2–0·3 cm. away from the surface and about 1·0 cm. wide, and secondly that the deposit shows concentric zoning. This paper is concerned with an explanation of these two phenomena.

Since all the neck canals of the perithecia immersed in the stroma are more or less at right angles to the surface, it is to be expected that the asci which protrude through the ostioles will discharge their spores roughly in this direction. If all the spore projectiles were of the same size and if all the asci burst with the same force one might suppose that the spore deposit would consist essentially of a line parallel with the edge of the stroma. However, because of variation in the force of ascus discharge and in the size of the ascospores some blurring of the line would be expected, but not to the extent of producing a wide band.

The probable explanation becomes apparent when a spore deposit is examined microscopically. For this purpose an overnight accumulation is too dense and it is best to look at one which has been formed at night during the course of about half an hour. It is then seen that there are many single ascospores in the deposit, and also groups ranging from two

to eight; single spores, and groups of eight being, perhaps, the commonest.*

We see then that the ascospore projectiles from a discharging *Daldinia* consist normally of from one to eight spores. It is very unusual to find an ascus with less than eight spores, so where a projectile consists of fewer than eight it has probably been formed from part only of the ascus contents, or, in other words, the contents have become separated into more than one projectile and, indeed, often eight are formed. No doubt there is a break-up of the spore jet so that droplets containing less than eight spores are formed.

With microscopic spherical projectiles the distance (d) of discharge with a given initial velocity is expressed by $d = Kr^2$, where r is the radius of the projectile and K a constant. We would expect, therefore, that 8-spored projectiles would be shot farther than those consisting of a single spore. This is easily confirmed by looking at the spore deposit. On the outer boundary only groups (mostly 8-spored) are to be seen, whilst on the inner boundary there are great numbers of single spores, although a few groups of spores of the other sizes are also present (Pl. 14 *b*, *c*).

We may suppose that there are eight possible spore projectiles with volumes 1, 2, 3, 4, 5, 6, 7 and 8, neglecting the fluid associated with the spores or assuming that the amount is proportional to the number of spores present. If we assume that these projectiles are spherical, which is not in fact so but may not be very far from the truth, then the relative distances of discharge with a given initial velocity are determined by the squares of the cube roots of the numbers namely: 1·00, 1·59, 2·08, 2·52, 2·92, 3·30, 3·66 and 4·00. The result would be a broad band of spores parallel with the surface of the stroma and at a certain distance from it, and if this distance is one unit then the band will have a breadth of three units. This is, in fact, very much the picture that is actually obtained, the white zone between the stroma and the deposit being about a third, or perhaps a quarter, of the width of the deposit itself.

Now if each of the eight sizes of projectile were discharged to its now characteristic distance, the deposit would have eight concentric zones, the outer ones becoming progressively closer together. In fact the deposit is usually zoned but the sharpness of the zoning varies considerably. Further the number of dark zones is always less than eight—usually only three or four. Nevertheless, it seems likely that the phenomenon of zoning is due to the occurrence of projectiles of a limited number of sizes with some of the zones tending to run together. Very probably some sizes of projectile are commoner than others so that certain zones may be emphasized at the

* There are also occasional clumps each composed of a large number of spores. These seem to arise in the following manner. Spore tendrils may occur in *Daldinia*. When this happens an ascus ruptures at the ostiole and its contents, instead of being violently discharged, simply ooze out. The next ascus follows suit adding its eight spores below the first eight, and if this goes on repeatedly, a spore tendril several millimetres long may be produced. Sometimes, shortly after a tendril has begun to form, an ascus succeeds in exploding with sufficient force not only to discharge its own eight spores, but also to carry away with them the accumulated spore mass of the incipient tendril. Such masses are normally shot only a very short distance—rarely more than a millimetre or two.

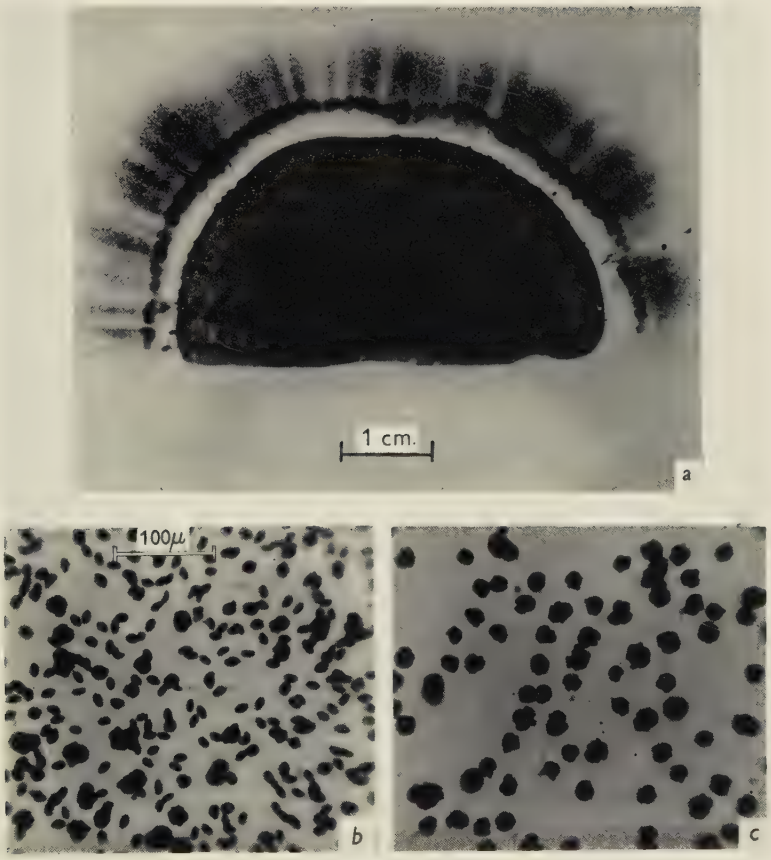
expense of others. Again the distance to which spore projectiles of each size are shot will certainly vary about a mean position and with the shorter range the smaller projectiles might be expected to show less absolute amplitude of variation than the larger ones with the greater range. Thus the inner zones would be expected to be more clearly defined and the outer ones more blurred.

EXPLANATION OF PLATE 14

a: spore deposit accumulated overnight around a thick median slice of *Daldinia concentrica*.

b, *c*: photographs taken under low power of the inner edge (*b*) and the outer edge (*c*) of a thin spore deposit, obtained after about half an hour, from a thick slice of *Daldinia*.

(*Accepted for publication* 28 *September* 1955)



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Trans. Brit. mycol. Soc. **39** (3), 381–390 (1956).

PROCEEDINGS, 1955–6

MEETINGS

Meeting held at Birkbeck College, London, W.C. 1, at 11.0 a.m., 14 October 1955. The President, Dr L. E. Hawker, in the Chair.

Diseases of fruit and hops

P. W. Talboys. Mechanism of *Verticillium* wilt tolerance in the hop.

The following provisional conclusions on the nature of Wilt tolerance in the hop have been derived from comparative studies of Wilt-sensitive and tolerant commercial varieties:

It has been shown that *Verticillium* metabolites produced in culture induce desiccation and leaf necrosis in hop shoots, irrespective of the Wilt-sensitivity of the variety and of the pathogenicity of the fungal strain.

Grafting experiments have shown that symptom intensity is determined by the host-parasite relationship in the root and is not influenced by the stem.

Two phases of disease development are postulated: (1) the *determinative* phase, in which conditions leading to symptom development are determined and established; and (2) the *expressive* phase, in which the conditions of vascular invasion determined and established in phase (1) develop, and become apparent in terms of visual symptoms.

Acute disease in a sensitive variety is associated with cell-wall lignification and lignituber development in the epidermis and cortex of the root in response to the presence of the fungus, invasion of the endodermis and pericycle, extensive entry of the pathogen into the xylem, and rapid colonization of the vascular system, with little tylosis. Secondary wood production does not appreciably exceed that in a healthy plant. It is probable that symptoms are primarily toxin-induced.

Mild disease caused by a virulent strain in a tolerant variety is also associated with cell-wall changes and lignituber development, but in addition there tends to be obstruction of the fungus at the endodermis. Invasion of the pericycle and xylem is consequently reduced, and it is probable that the very extensive tylosis which occurs limits the rate of vascular colonization. Greatly increased secondary wood production compensates for the vascular obstruction caused by the tyloses. In this syndrome leaf symptoms may be induced by tylosis of the leaf trace vessels rather than by toxic action.

It is tentatively suggested that the attainment of conditions of infection which determine whether or not extensive tylosis will occur constitutes the final stage of the determinative phase.

A. T. K. Corke. The shoot infection phase of *Gloeosporium* spp. on apple.

Recent heavy losses of stored apples led to an investigation being started in 1953 into the part played by the bitter rot fungi. Because of the importance of wood infections as sources of inoculum, a study of seasonal variations in the susceptibility of shoots of Cox's Orange Pippin to *Gloeosporium* attack was begun at Long Ashton in June.

Ten inoculations with mycelium and twenty with spore suspension were made monthly for 13 months, using one row of 8-year-old dwarf-pyramid trees for *G. album* and one for *G. perennans*.

Measurements and records of the cankers induced showed that inoculations with mycelium of *G. perennans* were successful throughout the year. Cankers induced by autumn or spring inoculations grew most rapidly and for the longest periods. No growth was observed after about the September following inoculation. Low temperatures in January and February caused a reduction in the rate of growth, but the rate was generally inversely proportional to the level of activity of the host.

Inoculations with spores emphasized that *G. perennans* was weakly parasitic and only able to grow well when host resistance was low.

Sporulation on artificially induced cankers began about April on older cankers, and in May or June on those initiated after January, and continued under favourable conditions until mid-winter.

Inoculations with *G. album* were generally unsuccessful, although some made in the period July–September resulted in short-lived, non-sporing cankers. This suggests that this species is able to survive for some time in wound tissue and to invade living tissues to a very limited extent during host dormancy.

It seems reasonable to conclude from these results that infections due to the invasion of wound tissue by *G. perennans* would generally be more likely to follow winter than summer pruning, and that it is only infections occurring between September and May which can become established and provide inoculum in the following summer.

K. L. Edney. The rotting of apples by *Gloeosporium* spp.

In recent years there has been considerable rotting of stored apples due to *Gloeosporium* spp. The variety most badly affected is Cox's Orange-Pippin (Cox's), but instances of losses of other varieties have been recorded. Two species are commonly found to be responsible for this wastage, *G. perennans* and *G. album*, the former very much more frequently than the latter. Rotting in most instances starts from the lenticel but it may also start from cracks.

Lenticel rotting of picked fruit by *G. perennans* may be produced by the application of a spore suspension. Using this technique, considerable variations in susceptibility to rotting of different samples of Cox's has been demonstrated. Seasonal variations in susceptibility of fruit from the same orchard have also been observed. Comparison of different varieties has not shown that Cox's are consistently more susceptible to infection than other varieties but intra-varietal variations from year to year make inter-varietal comparisons difficult.

Lenticels which are closed to gaseous exchange have been found to be very resistant to penetration by the fungus. The optimum temperature for invasion of Cox's by *G. perennans* is about 20° C., but rotting can still occur at 0° C.

As yet it has not been found possible to relate the extent of rotting in commercial samples of fruit to susceptibility or to the size of the spore load. Observations made in commercial orchards which had experienced high losses of stored fruit showed that in most instances tree infections could be found. Many instances of infection of winter pruning cuts have been encountered and damage by woolly aphis has often been found to be the means of entry of the fungus. The release of spores by cankers of *G. perennans* produced by inoculation has been observed to continue for periods of up to 18 months. This fungus has been grown on detached leaves of Cox's, and it has been found on dead leaves in the orchard after leaf-fall. Direct measurements of the numbers of spores reaching fruits have not been made. A rough estimate of the extent of relative infection can be obtained by comparing the wastage in samples of fruit which have been loaded with spores with comparable unloaded samples.

Some reduction of rotting has been obtained by the use of orchard sprays and by storing in an atmosphere of 3% oxygen and 5% carbon dioxide.

The factors which contribute to susceptibility are also at present not known.

This paper was prepared as part of the programme of the Food Investigation organization of the Department of Scientific and Industrial Research.

C. H. Cadman. Modes of transmission and field spread of raspberry viruses.

Excluding *Rubus* stunt virus, which is transmitted by leaf hoppers and not widely distributed in Britain, viruses that infect raspberries fall under two heads: (*a*) those mechanically but not aphid transmissible; and (*b*) those transmissible by aphids but not so far by mechanical means.

Viruses of category (*b*) cause the mosaic diseases ubiquitous among cultivated and wild raspberries and are responsible for the deterioration in vigour of commercial crops. The principal aphid vector in Britain is *Amphorophora rubi*; *Aphis idaei* is a vector of only one of the less important viruses. Field and glasshouse experiments have shown that raspberry varieties differ widely in degree of resistance to infection with viruses transmitted by *Amphorophora rubi* and that all are much more resistant than the North American black raspberry, *Rubus occidentalis*. Rates of spread of these viruses in the field are slow. In experimental plots of Lloyd George there was an annual twofold increase in numbers of plants infected with vein-banding virus. Control of aphids by insecticidal sprays reduced this increase to one tenth.

Viruses of category (*a*) have been isolated from raspberry affected by leaf-curl disease in Scotland and from diseased raspberry plants from two localities in England. These viruses are mechanically transmissible from raspberry to a wide range of herbaceous plants in which they cause ringspot diseases. Similar viruses have been isolated from naturally infected weeds and sugar beet. None of these is transmitted by raspberry aphids and an aerial vector seems improbable. Field and glasshouse experiments have shown that ringspot viruses infecting sugar beet are soil-borne and it seems likely that those infecting raspberry spread in the same fashion.

Epidemics of leaf-curl disease in Scottish plantations are not uncommon, though normally there is an annual twofold increase in infected plants. Most commercial varieties are either killed or greatly weakened by these ringspot viruses though Lloyd George and Malling Landmark seem immune.

A. F. Posnette. The heat therapy of virus diseases of fruit crops.

Heat treatment has been applied to those fruit plants in which virus infection commonly occurs throughout a variety: strawberry, apple, cherry and gooseberry.

Only 'whole plant' treatment has been successful; heating graftwood has impaired its ability to unite with, or to survive on, rootstocks without necessarily inactivating the viruses concerned. Complete inactivation has occurred of strawberry viruses, 1, 3 and 4 (Prentice, 1952), and of apple mosaic, when plants were maintained at about 37° C. for periods of 10–16 days for strawberries and 4 weeks for apples. Results with gooseberry vein-banding virus are promising, but the experiments are too recent for definite conclusions to be drawn. The effect of heat treatment on strawberry virus 2 (yellow edge) and on rusty mottle of cherry is transitory, and no plant material has been freed permanently from these viruses.

The highest proportion of virus-free strawberry plants was not always obtained with the longest treatment at 37° C., and it seems probable that damage to the plant is connected in some way with virus inactivation. Heat may act indirectly on virus multiplication through an effect on host metabolism. Alternatively, variations in the temperature at different sites in the heat frame may have caused more rapid virus inactivation in some plants than in others treated for a longer time.

R. W. Marsh. Spraying treatments against *Botrytis cinerea* on strawberries, and the effect of fungicide residues on the processed fruit.

Replicated plots of Auchincruive Climax strawberries were sprayed on 17 May, 1 June and 17 June 1954 with the following fungicides: 0·4% thiram, 0·2% salicylanilide, 0·2% tecnazene, 0·25% captan and 0·125% captan. Picking was done on seven dates from 9 to 27 July. Wet, cool weather favoured fruit attack by *Botrytis cinerea* which became epidemic after 19 July.

The salicylanilide and tecnazene sprays entirely failed to check the spread of infection: 0·125% captan controlled the disease only during the period of light attack: 0·25% captan and 0·4% thiram reduced the number of *Botrytis*-infected fruits to approximately one-third of that in comparable unsprayed plots.

Spray residues on the captan-sprayed fruits at the time of picking ranged from 7 to

19 p.p.m. These deposits did not affect the flavour or the ascorbic-acid content of the fresh strawberries.

Canned fruits from the plots treated with either salicylanilide or tecnazene showed no significant differences from unsprayed controls in flavour, aroma or appearance. Canned strawberries from the thiram-sprayed plots, tasted in November, were markedly tainted. A less noticeable, but significant, tainting was recorded in the canned fruit from plots sprayed with captan (0·125% or 0·25%). This was apparently due to a decomposition product of the fungicide, since no captan could be detected in the canned product.

No deleterious effect was noted in jams made from any of the sprayed fruits.

J. E. Crosse and R. S. Pitcher. Cauliflower of strawberry and related diseases.

The two most important symptoms associated with the infestation of field strawberries with nematodes of the genus *Aphelenchoides* are: (1) *alaminate leaves*, with short ($\frac{1}{2}$–1 cm.), more or less glabrous petioles, often browned superficially and tapered to a point representing an extremely reduced lamina; and (2) *cauliflower*, a symptom of the 'leafy gall' type.

Previous work, using strawberry runners growing in open pots, has shown that Cauliflower disease is due to certain strains of the bacterial pathogen, *Corynebacterium fascians*, in obligate association with the nematode *Aphelenchoides ritzema-bosi* (Crosse & Pitcher, *Ann. app. Biol.*, **39**, 475–486, 1952). Evidence that the alaminate leaf was caused by the nematode, however, was invalidated by the occurrence in plants inoculated with nematodes alone, of 'wild' strains of *Corynebacterium fascians* or of an organism closely resembling it.

To eliminate the bacterial factor, *Fragaria vesca* seedlings, grown aseptically in autoclaved soil or on an agar medium, were inoculated with nematodes surface sterilized beforehand in a sterilant containing ethoxy ethyl mercury chloride as the active agent (Crosse & Pitcher, *Rep. E. Malling Res. Sta.* (1952), pp. 138–40, 1953). Under these conditions alaminate leaves were produced by nematodes in the absence of all other micro-organisms.

As on runner plants, cauliflowers resulted on the seedlings only when the nematode and bacterium were co-inoculated. In contrast to runner plants, however, where inoculations with the bacterium by itself have given consistently negative results, *Corynebacterium fascians* caused 'leafy galls' on seedlings in the absence of the nematode. Although these were sufficiently like cauliflowers to confirm the bacterium as the main etiological agent in Cauliflower disease, there were differences which suggested that in Cauliflower disease the activity of the bacterium, or the response of the host plant to the activity of the bacterium, was modified by the presence of the nematode.

In the light of the previous work, the experiments with *Fragaria vesca* seedlings indicate that the most common symptom of nematode infestation of field strawberries, namely alaminate leaves followed by the death of these structures and the formation of 'open-centre' plants, is due to the nematode. The presence of nematodes is necessary for cauliflower, although this symptom is mainly caused by the bacterium. In this case the nematode probably acts primarily as a vector, carrying the bacterium into the meristematic areas of the plant normally inaccessible to it because of their tight enclosure in stipules and leaf bases.

R. V. Harris. Recent progress and future prospects.

Meeting held at Birkbeck College, London at 11.0 a.m., 7 January 1956. The President, Mr W. D. Graddon, in the Chair.

Kaung Zan. Persistence and movement of *Phytophthora infestans* in soil.

These investigations were undertaken to find out how long a soil which has once been infested by sporangia of *Phytophthora infestans* can remain infective to potato tubers and in what form the fungus persists in soil.

Aqueous suspensions of sporangia are added to natural unsterilized sieved air-dried soils. Infectivity is tested by placing small samples of the soil on potato-tuber slices which are incubated at 20° C. in moist chambers. Presence of the fungus is shown by the growth of aerial mycelia bearing characteristic sporangia after 5–6 days. Infectivity can persist in the soil for 9–10 weeks, depending on the water content and temperature of the soil. The optimum water content is 15–20% of saturation, above or below this the time soil remains infective decreases. Infectivity is decreased by an increase in temperature. Various soil types, at pH 4·8–8·0, and different spore concentrations do not seem to affect the persistence of infection very much. It is improbable that infectivity persists in the soil as oospores or chlamydospores. The former do not occur freely in nature and chlamydospores cannot be formed, as hyphae of *P. infestans* will not grow in unsterile soil.

When zoospores instead of sporangial suspensions are used, infectivity stops after 2 weeks. It is therefore impossible to assume that infectivity persists as zoospores for long periods, as had been stated previously. Thus there are reasons to believe *P. infestans* persists in soils as sporangia or as germ tubes after their germination.

D. E. Etheridge. Occurrence of *Coryne sarcoides* with heartrot fungi on spruce in Alberta, Canada.

A fungus, identified as the imperfect form of *Coryne sarcoides* (Jacq.) Tul., has been found with decay in living spruce in Alberta, Canada. What is believed to be the same fungus has also been found with decays in other coniferous and deciduous tree hosts in every major forest region from Newfoundland to British Columbia. This appears to be the first record of *C. sarcoides* occurring with decays in living forest trees, and the first record of its occurrence on coniferous hosts (for previously published reports see *Bi-monthly Progr. Rep. Dep. Agric. Can. (Div. For. Biol.)*, **10** (4), 3–4, 1954; **11** (6), 3, 1955).

On the basis of decay studies of spruce made in Alberta during 1952 and 1954, approximately 15% of the typical decay infections that were sampled yielded this fungus. *C. sarcoides* was found associated with nearly all the principal decay-producing fungi on this species, but occurred more frequently with the fungi producing the basal rots. Except in some cases where a pink stain has been associated with the infections, this fungus does not produce a characteristic decay.

Although *C. sarcoides* normally occurs with decay-producing fungi, successful colonization by this fungus can occur independently of decay. Relatively large numbers of cultures of *C. sarcoides* were obtained from the heartwood of healthy trees (7·1% of the samples examined), and from apparently normal wood samples from infected trees (17·8%). The presence of *C. sarcoides* in healthy trees suggests that it might be a pioneer organism in the succession of fungi that attack living trees, but the exact nature of its relationship with other fungi has yet to be determined.

Preliminary investigations to determine whether or not *C. sarcoides* is capable of causing decay (loss-in-weight of wood substance) have to date yielded negative results, but studies are being continued with the fungus to obtain information regarding the nature of its growth on wood, and its effect on the activity of decay-producing fungi. In comparative physiological studies with heart rot fungi, it has been found that some isolates of *C. sarcoides* possess enzymes, viz. cellulases and polyphenol oxidases, similar to those which characterize the fungi causing white-rots in wood.

(Since this paper was presented, the occurrence in England of *C. sarcoides* (*Pirobasidium sarcoides* (Jacq.) Hoehn.) with decays in living trees of beech, Sitka spruce, and lodgepole pine has been established by Dr S. Batko, Mycologist, Forestry Commission, Alice Holt, Wrecclesham, Surrey.)

E. W. Buxton. Physiologic races of *Fusarium oxysporum* on peas: mechanisms of variation in host and parasite.

Three physiologic races of *Fusarium oxysporum* Fr. f. *pisi* (Lindf.) Snyder & Hansen, which causes pea wilt in England, have been identified. Some of the variability of the fungus, both in cultural characters and in pathogenicity, is caused by heterokaryosis between pairs of cultural variants and between pathogenic strains. To study heterokaryosis between pathogenic races, mutants having different nutritional requirements were made by exposing conidia to ultraviolet radiation. Most of such mutants were less virulent than wild-types; heterokaryons between them had virulence equal to that of the wild-types. Three conidia in 10^8 from a heterokaryon formed between physiologic races 1 and 2 produced segregant and recombinant progeny, suggesting that the three were diploids. The ability to infect specific pea varieties was recombined with nutritional deficiencies, colour differences and with different abilities to tolerate growth products of Actinomycetes. This system of genetic variation in *F. oxysporum*, which has no perfect state, is similar to the 'parasexual cycle' described by Pontecorvo in *Aspergillus* and *Penicillium* species.

Root exudates from three pea varieties that act as differential hosts for the three physiologic races delayed or suppressed the germination of spores of races to which the varieties are resistant but not of races to which they are susceptible. The ability of exudates to affect germination decreased as the roots grew older, although a temporary increase in activity occurred when side roots first emerged from the main root. When the exudates were concentrated and placed in porcelain cylinders on agar media, they inhibited the growth of those races which they resist but not those to which they are susceptible. Sectioning roots of inoculated plants showed that races incapable of causing wilt in specific varieties were checked in the outer layers of the cortex and never reached the vascular tract. This differentiation was again most pronounced in the early stages of the plant's growth. Extracts of stem-base lesions caused by *Fusarium solani* f. *pisi* and of stem-bases inoculated with non-pathogenic *F. solani* suppressed the germination of spores of *F. oxysporum*, whereas extract from uninoculated stem-bases did not.

Miss Irene M. Wilson. Some marine fungi on wood.

Marine fungi were collected from wood blocks attached at various levels on the shore at Aberystwyth. Blocks near L.W.M. in moist situations produced most species. All fungi obtained belonged to the Pyrenomycetes or Fungi Imperfecti.

Three typical genera of marine Pyrenomycetes were described, *Ceriosporopsis*, *Halophiobolus* and a genus still undescribed. These genera have certain common characteristics, not necessarily shown by marine Pyrenomycetes on seaweeds: (1) they are formed immersed in the substrate and subsequently exposed by erosion, (2) the asci deliquesce and liberate the spores into the perithecial cavity, (3) the spores have floating, entangling and adhesive devices, (4) there is no seasonal fruiting period and the spores germinate without a resting period, but chlamydospores are frequently formed as a vegetative resting stage, (5) all the species can utilize all the elements in the wood but show preferences for certain constituents.

The mycelium of marine Pyrenomycetes in wood was shown by Barghoorn in 1944 to consist of a coarse mycelium running through the wood elements and rays with boreholes through the walls, and a fine mycelium within the walls of the wood elements themselves. All the species described soften the surface layers of the wood and thus aid its erosion by the sea and are mainly important in the early stages of decay. They are succeeded by marine Crustacean borers with which only *Ceriosporopsis halima* and an undescribed *Halophiobolus* species can compete, and these feebly.

Some Fungi Imperfecti were also illustrated including *Helicoma maritimum* Linder, *Speira pelagica* Linder and some unnamed species including types with 3- and 4-armed conidia.

S. A. Hutchinson and M. M. Fahim. Fungal diseases of bracken gametophytes.

The germination of bracken spores and the growth of the prothalli on mineral media is stimulated by the presence of fungal contaminants in the cultures, and many spores do not germinate or do not give rise to normal prothalli in pure cultures. The proportion of such spores increases during the storage of the spores in a dormant condition.

The prothalli are generally susceptible to attack by a wide variety of pathogenic fungi, but individual prothalli and groups of cells within prothalli vary in their susceptibility. In some cases the attack of the fungus may be restricted to an area round the site of inoculation, forming a localized lesion. A detailed study of attack by *Botrytis cinerea* has shown that the containment of the fungus within localized lesions is due to changes in the host cell walls immediately around the invaded area, and not directly to changes in the host cytoplasm. These changes are the result of an interaction between the living cells of the host and a metabolite secreted by the fungus, and they are shown macroscopically by the formation of an unidentified brown pigment in the host cell walls. The changes occur more rapidly in plants which have been exposed to long periods of illumination before and after inoculation than in those exposed to short periods. They are also affected by changes in other external conditions.

Miss S. Hepple. The distribution of *Mucor ramannianus* in podsolized soil.

In a humus podsol under *Pinus sylvestris* in Delamere Forest, *Mucor ramannianus* was found to be almost completely restricted to the B_1 horizon where it was the dominant organism. The distribution was based on dilution plate counts using acid Dox agar. Warcup and dilution plates using a series of other media gave similar results.

This is an example of the occurrence of a typical Phycomycete in a seemingly anomalous situation.

Direct observation showed that most of the mycelium was dead and that colonies obtained during isolation developed from chlamydospores. Hyphae in the soil were unlike those of *M. ramannianus* in pure culture, but when *Mucor* was grown on soil extracts and on pine-needle extracts a mycelial type like the soil hyphae was obtained. Dissection of soil aggregates showed that the fungus was associated with tiny root fragments in an arrested state of decomposition, that is, the cellulose and lignin portions remained but had fallen apart. *M. ramannianus* could cause decomposition up to this stage. Further breakdown was precluded because no other organisms were present. It was suggested that their absence was due to a raised concentration of carbon dioxide in the lower soil layers.

The position of *M. ramannianus* in this soil is, therefore, not anomalous but represents the first step in the normal succession during the decomposition of roots, the later stages of which are blocked.

Meeting held at the London School of Hygiene and Tropical Medicine at 10.30 a.m., 17 March 1956. Dr M. Ingram in the Chair.

M. Ingram. Introduction.

Some of the main points of interest about the yeasts, as a group, were reviewed to show how the individual contributions to the symposium fitted into a general picture. Subjects mentioned were: the heterogeneity of the group and relations to diverse fungi; sporulation and heterothallism; formation of pseudomycelium; fermentation and adaptation; cytology and genetics, and the use of genetics to elucidate biochemical behaviour such as fermenting powers; the growth of industrial yeasts; inter-relations among carotenoid-producing yeasts; the ecology of the yeasts in general and of the osmophiles in particular.

F. W. Beech. Some practical problems of yeast identification.

Microbiologists studying yeasts usually wish to identify them only as a preliminary to further work, rather than to devise new systems of classification. The classical morphological and biochemical tests were each examined in detail and anomalies and difficulties, caused by lack of knowledge of the nutrition of yeasts in all genera, pointed out. Results obtained with a large number of yeasts, isolated originally from apple juice and cider, were used as illustrations. Methods suitable for the separation of strains within a species group were also discussed, together with others devised by the author, that may also be used for this purpose. In particular, the examination of culture fluids by chromatographic methods appears to be especially useful. Finally a method was described for summarizing results on marginally punched cards as an aid to yeast classification.

J. A. Barnett. Some unsolved problems of yeast taxonomy.

G. C. Ainsworth. Pathogenic yeasts.

Few yeasts are pathogenic and those that are characteristically infect animals. A number of important systemic mycoses of man and animals are caused by yeasts, while other similar diseases are caused by mycelial fungi which have a yeast-like parasitic phase. Yeast-like cells are one of the two characteristic forms assumed by fungi when parasitizing tissues of higher animals. Possible reasons for this and its bearing on mechanisms of pathogenicity were considered.

F. T. Last. The distribution of *Sporobolomyces* and *Tilletiopsis*.

Sporobolomyces and *Tilletiopsis* both belong to the Sporobolomycetaceae and produce spores which are discharged by the drop-excretion method typical of the Hymenomycetes. Concentrations of 490,000 and 197,000 *Sporobolomyces* and *Tilletiopsis* spores per cubic metre of air, respectively, have been recorded. *Sporobolomyces* spp. have been isolated from living and dead leaves of ferns, mono- and dicotyledons in Europe, Asia and America. The recorded distribution of *Tilletiopsis* is more restricted.

The distribution of these fungi on leaf surfaces is not haphazard, and like the rhizosphere, the 'phyllosphere' has a highly organized non-parasitic flora. By holding leaves contaminated with these fungi above agar, allowing the colonies to discharge spores which fall vertically and then incubating the agar, a mirror image of the distribution of these fungi on the leaf is obtained. The pattern of colonies simulates the leaf venation.

The numbers of *Sporobolomyces roseus* and *Tilletiopsis minor* colonies isolated from cereal leaves were related to the age of the leaf. Few colonies were isolated from young leaves but the numbers increased rapidly after leaves had lived half their lives. More colonies were isolated from the distal than from the proximal part of cereal leaves which die progressively from the distal end backwards.

The development of these fungi in the phyllosphere appears to be related to the physiological state of the leaf. Recently the development of colonies of the bacterium *Beijerinckia* on leaves of some tropical plants was shown to follow the same trends as *Sporobolomyces* and *Tilletiopsis* colonies but, unlike the latter, *Beijerinckia* may in addition be useful to the plant as it can fix nitrogen (Ruinen, *Nature, Lond.*, **177**, 220–221, 1956).

R. R. Fowell. The crossing of yeasts with low spore viability.

Certain yeasts, with desirable features of possible commercial value, cannot be readily hybridized with other yeasts because of the poor viability of their spores; this applies in particular to some brewery yeasts.

Low spore viability is often associated with poor sporulating ability. Hence it is essential to use a satisfactory sporulation medium, e.g. sodium acetate agar (Fowell, *Nature, Lond.*, **170**, 578, 1952), in order to obtain sufficient numbers of asci containing spores.

The normal method of obtaining spores by the dissection of asci, besides being time-consuming, may yield few or no viable spores. The evidence available suggests that the cause of low spore viability is largely genetical. Hence, there are three possible lines of attack on this problem: (1) improvement in the technique of spore isolation, (2) inbreeding directed towards the elimination of genetical factors with an adverse effect on spore viability, and (3) improvement in hybridization technique (Wickerham, *Nature, Lond.*, **176**, 22, 1955).

Isolation of spores as such is not necessary, because hydridization is conveniently effected by mass mating of haploid cultures derived from spores. Haploid cultures can be rapidly obtained, either by the isolation of intact asci or by selective heat treatment of sporulating cultures (Wickerham & Burton, *J. Bact.* **67**, 303, 1954).

Isolation of intact asci is justified when there is little possibility of more than one spore per ascus germinating to produce a haploid culture. In cases of doubt, single cells may be isolated from colonies developed by asci on malt agar films.

The selective heat-treatment method, first devised for *Hansenula*, has been successfully applied to *Saccharomyces*; it depends on the fact that spores are more resistant to heat than vegetative cells.

Inbreeding applied to a yeast with less than 1 % viable spores resulted in the production of two strains showing (1) much improved spore viability, and (2) enhanced mating activity among haploid cultures. These strains successfully replaced the parent yeast in hybridization studies.

J. White. Osmosensitivity and enzyme activity as functions of the environment in mass culture of yeasts.

Investigations over the last few years have demonstrated immense differences in the behaviour of yeasts grown under different cultural conditions. Bakers' yeast strains may be divided into two broad environmental types which are produced industrially in most yeast manufacturing operations. '*Non-incremental*' yeasts are produced by the growth of a yeast seed in an aerated wort which initially contains significant amounts of sugar, together with all the necessary mineral salts and vitamins to sustain growth and fermentation. In commercial practice such yeasts are used as seeding yeasts for the production of large quantities of '*incremental*' yeasts (the normal bakers' yeasts of commerce), which are grown by feeding the sugar-containing wort (and some of the requisite inorganic salts) by small increments over a sustained growth period into an aqueous suspension of the seed yeast, in the presence of violent aeration. By such means, alcoholic fermentation is suppressed and growth or *assimilation* is encouraged. Brewers' and distillers' yeasts are non-incremental yeasts.

There are very considerable differences in behaviour between incremental and non-incremental yeasts. When seeded into aerated sugar solutions of moderate osmotic concentration (e.g. 10% w/v D-glucose) containing all the mineral salts and vitamins requisite for growth, the non-incremental yeasts usually commence growth at a steady and logarithmically-increasing rate, giving the classical conception of exponential yeast growth. Incremental yeasts, however, often pass through a 'loss-phase' of growth (in which the yeast seed actually loses significant weight) before the yeast adapts itself to existence in the solution and becomes stabilized through a 'recovery phase' and ultimately commences growth in the 'exponential phase'. Carbon dioxide evolution occurs in the loss phase, although rate of gas production gradually increases through the loss to the recovery and into the final exponential growth phases.

Incremental (commercial bakers') yeasts usually produce carbon dioxide gas at a satisfactory rate in commercial media (e.g. doughs used in bread and bun production) even under conditions of high osmotic pressure (such media often contain large amounts of sugar and salts). Non-incremental yeasts usually exhibit considerable osmosensitivity and give poor gas-producing performances in such media. A peptide-like substance present in flour has been demonstrated to inhibit fermentation in media of high osmotic pressures to a far greater extent with non-incremental (osmosensitive) than with incremental yeasts.

When a commercial incremental yeast is seeded into an aerated sugar solution of moderate strength it rapidly loses its osmotolerant characteristics and passes through

an extremely osmosensitive phase. Towards the end of the fermentation a degree of stability is produced in the yeast, but the final crop is usually far more osmosensitive than the initial incremental seed. Osmosensitivity increases with increasing growth rate and is, undoubtedly, a function of the chemical constitution of the cell.

Yeast strains vary in their tendencies towards production of osmosensitivity and osmosensitivity increases as yeasts age after harvesting. Yeasts harvested from the active logarithmic growth phase of a fermentation in an aerated sugar solution usually exhibit strong autolytic tendencies in addition to considerable osmosensitivity.

Studies of the zymase, malto-zymase and invertase content of yeasts grown in solutions of various sugars (e.g. sucrose, maltose and glucose) have shown the enzyme content of yeasts to be a function both of the composition of the nutrient solution and of the stage of the fermentation at which the yeast is separated from the substrate. A cycle of considerable dynamic change in yeast composition is set in motion when yeast is allowed to ferment a sugar solution, both adaptive and constitutive enzyme systems being involved. Great variations in the enzyme content of the yeast crops are obtained, depending upon the stage of the fermentation at which the yeasts are harvested.

Miss M. P. Scarr. The micro-ecology of osmophilic yeasts.

Osmophilic yeasts occur in small numbers in a wide range of habitats where there are sugars in the medium. Defining osmophilic yeasts as those able to grow at concentrations of 45%, w/w, dissolved sugars and over, they are found in small numbers in fresh fruits, nectar, the exudates of trees and the sugar cane. After a 'drying-out' of the medium, these yeasts predominate as the osmotic pressure is then so high that only they can multiply. Such habitats are honey, syrups of all types which are homogeneous, and dried fruit, raw sugar, etc., where the yeasts are found in the film of syrup on the surface of the substance. The main species involved are *Saccharomyces rouxii*, *S. mellis*, *S. bisporus*, *Schizosaccharomyces octosporus*, *S. pombe*, *Hansenula anomala* and *Torulopsis* spp. Appreciable growth can occur at concentrations up to 75%, w/w, dissolved solids.

Meeting held at Queens' College, Cambridge, at 10 a.m., 11 April 1956. Dr G. C. Ainsworth in the Chair.

J. C. Gentles. A survey of foot ringworm in coalminers.

Dr Mary J. Marples. The ecology of *Microsporum canis* Bodin.

P. K. C. Austwick. Some problems in the diagnosis of ringworm in animals.

F. Mariat. Histoplasmosis in African monkeys.

The meeting was followed by an afternoon visit, by kind invitation of Prof. P. L. Pugh, to the School of Veterinary Medicine.

THE BRISTOL FORAY

8–15 September 1955

By F. B. HORA, *Foray Secretary*

For the fifty-first Autumn Foray, members were accommodated at Wills Hall, University of Bristol, by kind permission of the Vice-Chancellor, Sir Phillip Morris. We are very grateful to Sir Phillip for this permission and also to the Warden of the Hall, Mr Kendall, who made sure that we were very well looked after. Down House, in the same grounds as Wills Hall, was put at our disposal as a work room. Sixty-one members attended.

In my last Autumn Report I estimated an average Autumn Foray would yield some 250 species of *agarics*. In the present instance, only about 100 were found. This small number reflects the phenomenally dry summer. It was a great disappointment to the agaricologists (at least), as Dr Meinhard Moser had come over from Innsbruck as our foreign guest and we had hoped to show him a lot of *Cortinarius* species, a genus of agarics in which he has a special knowledge. As the lists show, only two species were found—neither of them exactly critical!

Attention may be drawn to the list of Uredinales. Judging by their numbers, they seemed little affected by the dry summer.

As full-day excursions, we visited the sand dunes and salt marsh at Berrow on Friday, 9 September, the Forest of Dean on 11 September, and Stinchcombe Hill in the Cotswolds on 13 September. As half-day (or optional full-day) excursions, Blaise Castle was visited on 10 September, Cleeve Combe (in Somerset) on 12 September, and Leigh Woods (just across the Clifton Suspension Bridge) on 14 September.

On 9 September, the Society was privileged to entertain the Vice-Chancellor and Prof. Macgregor Skene to dinner. A fine meal was rounded off by some excellent speeches.

In the evening of 11 September, Dr Ramsbottom and Prof. Ingold each introduced a splendid example of the art of 'shooting' films down a microscope. Prof. Ingold's film was concerned—appropriately enough—with spore discharge, and Dr Ramsbottom's with fungi which parasitize eel-worms—a particularly gruesome example of Nature 'red in tooth and claw'—but quite fascinating: one was almost mesmerized by the horror of it!

A short business meeting was held on the evening of 12 September, followed by a most erudite lecture by Dr Moser on the 'Taxonomy of *Cortinarius* and related genera'. This was illustrated by many of his magnificent water-colour paintings which were greatly admired.

In spite of the exceptionally dry summer (perhaps even because of it) some interesting finds and new records were made. In the Uredinales, Dr Manners found a new host each for *Melampsora euphorbiae* and *Puccinia*

agropyrina. In the Agaricales, I was very pleased to re-find *Rhodophyllus leptonipes* and have it confirmed by Dr Moser, who had himself already gathered it on the continent in the company of the author of the species, M. Henri Romagnesi. I found it for the first time last year (1954) at Reading. We should place it under the genus *Eccilia*, but I have refrained from so doing here, because I am sure this is not the place for making new combinations. *Mycena citrino-marginata* is here definitely recorded as a good British species. The late A. A. Pearson would not admit it, regarding it as 'a woodland form of *M. avenacea*'. However, frequent gatherings in the last year or two amply confirm American and continental authors who maintain it. Dr Moser added two new records to our agaric list with *Inocybe tabacina* and *Psathyrella orbitarum*—both only recently described from the Continent. Mention should be made of *Psathyrella pseudo-gracilis*. At present, I am unable to distinguish this macroscopically from *P. gracilis*, under which name it undoubtedly hides in our lists. Microscopically, however, it is at once separated by having facial cystidia with a *rounded* apex. In *P. gracilis*, they have a distinctly *pointed* apex, and my experience at the moment is that this species is the less common of the two.

I have queried *Paxillus filamentosus* because it was not possible with the material available to make a detailed examination. Dr Moser drew my attention to it. It is very close to *P. involutus*, but has shorter and more rounded spores and the habitat is under alders. A careful look-out should be kept for it, as it is not yet in our list. I have also queried *Boletus leoninus* which is a distinctly rare species. Two British specimens only were seen by A. A. Pearson (these *Transactions*, **32**, 271 (1949)). We found only one specimen, but the 'remarkably tomentose cap' noted by Pearson was plainly visible. The determination must remain in some doubt on account of the inadequate material. It is noteworthy that Pearson gathered his specimens also in an unusually dry year—1947, in the New Forest. *Coprinus erythrocephalus* was a good find. Pearson regarded the British record as doubtful, no doubt because there has in the past been confusion with *C. dilectus* Fr. Once the cap cuticle is examined, they are easy to separate. It is filamentous in the former but cellular in the latter.

On looking through the agaric list, one notices that the species are mostly small, or if medium-sized, then they tend to grow on wood—but not in the case of *Lactarius* and *Russula*. From his own experiments, Dr Moser told me that, generally speaking, small species as well as those of *Lactarius* and *Russula* are able to develop their fruit-bodies relatively rapidly; that is, as soon as conditions are favourable. Perhaps, then, the agarics that we saw may correspond to the so-called 'desert ephemerals' that occur amongst the Flowering Plants. Wood-inhabiting species ai well known to occur in dry weather. They were, for example, a characteristic of the last Oxford Foray.

A special expression of my thanks must go to Dr Lilian Hawker for her help in organizing the Foray and for acting as local secretary.

We are all very indebted to Dr Moser for his learning, charm and the readiness with which he answered our many questions.

For help in supplying records I wish to thank Dr Agnes Brown and Miss Grace Waterhouse, Mr Peter Austwick and Mr Howard, Dr Manners,

Dr O'Connor, Mr P. D. Orton and Mr J. T. Palmer, Dr Parker-Rhodes, Dr J. Ramsbottom, Mr T. J. Wallace and Dr Webster. For nomenclature see these *Transactions*, **38**, 185 (1955).

List of Species

B. = Berrow sand dunes; *Bl.* = grounds and woods of Blaise Castle; *C.* = Cleeve Coombe; *D.* = Forest of Dean, including area around Speech House; *L.* = Leigh Woods; *S.* = Stinchcombe Hill; *W.* = grounds and woods of Wills Hall. Voucher material: [1] = in herb. P.K.C. Austwick; [2] = in herb. F. B. Hora; [3] = in herb. J. T. Palmer; [4] = in herb. University of Sheffield. † = new British record.

MYXOMYCETES

Arcyria denudata, *B.*, *S.*
Ceratiomyxa fruticulosa, *L.*
Fuligo septica, *Bl.*
Leocarpus fragilis, *Bl.*
Lycogala epidendrum, *D.*, *L.*
Physarum nutans, *S.*
Stemonitis fusca, *Bl.*
Trichia affinis, *L.*

PHYCOMYCETES

Bremia lactucae, on *Lactuca muralis*, *Bl.*
Peronospora alta Fuckel, on *Plantago major*, *Bl.*

EU-ASCOMYCETES

Pyrenomycetes

[4]Chaetosphaeria phaeostroma, on *Fagus*, *S.*
Claviceps purpurea, on *Agropyron*, *B.*
Cymadothea trifolii, on *Trifolium fragiferum*, *B.*
Erysiphe cichoracearum, on *Arctium lappa*, *S.*; graminis, *S.*; polygoni, on *Circaea lutetiana*, *Bl.*, *W.*, on *Polygonum aviculare*, *Bl.*, *W.*, on *Ranunculus acris*, *S.*; tortilis, *Bl.*, on *Cornus sanguinea*, *S.*; umbelliferarum, on *Heracleum* sp., *Bl.*, *C.*
[4]Eutypa flavo-virens, on *Fagus*, *S.*
[4]Hypoxylon fragiforme, on *Crataegus*, *Bl.*, on *Fagus*, *S.*; fuscum, *S.*
[4]Lasiosphaeria hirsuta, on *Fagus*, *S*; [4]ovina, on *Fagus*, *S.*
[4]Leptosphaeria rubelloides, on *Arrhenatherum elatius*, *Festuca arundinacea*, *Melilotus altissima*, *Scirpus maritimus*, *B.*
Mycosphaerella rumicis, *Bl.*
[4]Pleospora vagans, on *Agropyron junceiforme*, *B.*
Podosphaera oxyacanthae, *Bl.*
Sphaerotheca humuli, on burdock, *Bl.*, *var.* fuliginea, on *Taraxacum officinale*, *W.*; pannosa, *S.*
Xylaria hypoxylon, on *Fagus*, *S.*

Discomycetes

Fabraea ranunculi Karst., on *Ranunculus repens*, *Bl.*, *C.*, *S.*
Helotium virgultorum, *Bl.*
Hyaloscypha dematiicola (conidial), Ash Stack Grove.
Mollisia cinerea, *Bl.*, *D.*
Nectriopsis aureo-nitens (Tul.) Maire, on *Pinus* bark, *D.*
Pseudopeziza medicaginis, on *Medicago lupulina*, *S.*; trifolii, on *Trifolium pratense*, *S.*
Rhytisma acerinum, *Bl.*, *C.*, *S.*
Trochilia ilicis (Chev.) Rehm, *B.*, *D.*

HETERO-BASIDIOMYCETES

Auriculariales

Auricularia mesenterica, on elm stump in brackish water, *B.*

Tremallales

Exidia nucleata (form lacking 'core' of calcium oxalate = *Tremella hyaline* Pers.), on ash twig, *D.*
Protohydnum lividum, not sporulating, on bark of beech log, *S.*
Sebacina ?fugacissima, on stick of *Hippophae*, *B.*; grisea (Pers.) Bres., on wood, *Bl.*

Tulasnellales

Tulasnella ?violacea (J. Ols.) Juel, on birch stick, *D.*

Calocerales

Calocera cornea, *D.*
Dacrymyces stillatus *sensu* Martin (non Bourd. & Galz.), on beech bark, *D.*
Femsjonia luteo-alba, on dead branches of birch, *D.*

Uredinales

Coleosporium petasitis, on *Petasites* sp., *L.*; senecionis, *S.*, on *Senecio vulgaris*, *C.*; sonchi, on *Sonchus arvensis*, *B.*, *S.*; tussilaginis, on *Tussilago farfara*, *B.*
Cumminsiella mirabilissima, on *Mahonia* sp., *W.*
Kuehneola uredinis, on *Rubus fruticosus*, *C.*, *L.*, *S.*
Melampsora euphorbiae, on *Euphorbia paralias*, *B.*
Milesia polypodii, on *Polypodium*, *B.*
Phragmidium mucronatum, on *Rosa* sp., *C.*; violaceum, on *Rubus fruticosus*, *B.*, *C.*
Puccinia agropyrina, on *Agropyron junceiforme* and *A. repens*, *B.*; asperulae-odoratae, *S.*; brachypodii, on *Brachypodium sylvaticum*, *G.*, *S.*; buxi, on *Buxus sempervirens*, *C.*; caricina, on *Carex pendula*, *Bl.*, *L.*, on *Carex* sp., *B.*, *S.*; chondrillae, on *Lactuca muralis*, *S.*; circaeae, on *Circaea lutetiana*, *Bl.*, *C.*, *S.*; cnici, on *Cirsium vulgare*, *L.*; coronata, on *Arrhenatherum elatius*, *C.*, *L.*, *S.*, on *Festuca ovina*, *B.*, on *Holcus mollis*, *B.*; epilobii, *C.*, *S.*; glechomatis, on *Glechoma hederacea*, *B.*; leontodontis, on *Leontodon* sp., *S.*; menthae, on *Mentha aquatica*, *B.*; phragmitis, on *Phragmites communis*, *B.*; poarum, on *Tussilago farfara*, *S.*; pulverulenta, on *Epilobium hirsutum*, *Bl.*, *C.*; saniculae, on *Sanicula europaea*, *S.*, sessilis, on *Phalaris arundinacea*, *Bl.*; sonchi, on *Sonchus arvensis*, *B.*, *C.*; taraxaci, *Bl.*, *S.*, on *Taraxacum officinale*, *B.*; violae, on *Viola canina*, *B.*, on *V. hirta*, *C.*, on *V. riviniana*, *S.*
Pucciniastrum circaeae, on *Circaea lutetiana*, *Bl.*, *C.*, *S.*; epilobii, on *Chamaenerion angustifolium*, *C.*, *S.*
Uromyces dactylidis, on *Dactylis glomerata*, *B.*, *Bl.*, *S.*; fabae, on *Lathyrus pratensis* and *Vicia cracca*, *B.*; lineolatus, on *Oenanthe fistulosa* and *Scirpus maritimus*, *B.*; loti, on *Lotus corniculatus*, *C.*; pisi, on *Lathyrus pratensis*, *C.*; polygoni-aviculare, on *Polygonum aviculare*, *W.*; ramicis, *Bl.*; trifolii, on *Trifolium fragiferum*, *B.*

HOMO-BASIDIOMYCETES—Hymenomycetes

Aphyllophorales

Porohydnineae

Acia stenodon forma, on maple twigs in water, *S.*; uda, on wood, *S.*
Corticum centrifugum (Lév.) Bres., on *Pleurococcaceae* on bark of beech sticks, *D.*; (?) frustulosum Bres., on exposed root of oak, *Bl.*; lacteum Fr. (non Bourd. & Galz.), on sycamore bark, *S.*; microsporum, on dry basidiocarp of *Ganoderma applanatum*, *Bl.*; mutabile Bres., on bark of pine twigs under bracken, *C.*; punctulatum Cke., on oak bark and waterlogged birch sticks, *D.*; sambuci, on *Sambucus*, *Bl.*; subcoronatum, on rotten pine wood, *C.*; submutabile v. H. & L., on dead bracken petioles, *D.*; sulphureum Pers. non Fr., on moss and sticks, *C.*; ?umbratum Bourd. & Galz., on bark of *Hippophae*, *B.*; vagum, on rotten pine wood, *C.*

Cyphella [1]cupula, on stem of *Phragmites*, *B.*; *var.* flavescens, on dead *Epilobium* stem, *D.*
Daedalea confragosa, on small stick, *Symonds Yat*; quercina, on oak stump, *D.*, on worked wood, *Symonds Yat*, *S.*
Fistulina hepatica, on oak, *Bl.*, *D.*, *L.*, *W.*
Fomes annosus, on conifer, *Bl.*; ferruginosus, *D.*; igniarius *var.* nigricans, on willow, *B.*; ulmarius, *Bl.*; on elm, *D.*
Ganoderma applanatum, on beech and *Prunus laurocerasus*, *Bl.*; on dead wood, *D.*
Gloeocystidium contiguum (Karst.) Bourd. & Galz., on bark of alder, *B.*; pallidum (Bres.) v. H. & L., on twig of *Rhododendron*, *Bl.*; pertenue Karst. (?young stage of *G. praetermissum*), on *Sambucus*, *Symonds Yat*; tenue (Pat.) v. H. & L., on rotten wood of *Hippophae*, *B.*
Grandinia [1]farinacea, *B.*; helvetica, juvenile stage on larch cone, *D.*; mature stage on larch twig, *S.*
Hymenochaete corrugata, on hawthorn branch, *C.*; fuliginosa (Pers. ex Fr.) Lév., on hazel stick, *C.*; rubiginosa, on Crataegus, *C.*
Merulius [1]corium, *D.*
Mucronella aggregata, on rotten beech log, *D.*
Odontia papillosa, on yew branches, *C.*
Peniophora cinerea, on maple, *C.*, on ash, *Symonds Yat*; leprosa, on larch, *Bl.*, on beech, *D.*, on ash, *S.*; longispora, on rotten and waterlogged sycamore log, *S.*; nuda, on sycamore, *Bl.*, on beech and ash, *S.*; pubera, on bark of *Rhododendron*, *B.*, on beech, *S.*; quercina, on oak branches, *C.*, on beech, *S.*
Polyporus adustus, on beech log, *S.*; albidus, on fir wood, *D.*; betulinus, *Bl.*; caesius, *Bl.*, *C.*; chioneus, *C.*, *D.*; dryadeus, on *Quercus ilex*, *Bl.*; giganteus, *D.*, on *Q. ilex*, *Bl.*; hispidus, on living tree, *Bl.*; schweinitzii, on living tree, *D.*; [1]stipticus, on *Picea excelsa*, *Bl.*, *D.*; squarrosus (old), *Bl.*; sulphureus, *Bl.*, on yew trunk, *C.*, on living tree, *D.*
Polystictus hirsutus, on beech stump, *S.*, pubescens, on oak stump, *S.*; versicolor, *B.*, *D.*, on sycamore and *Prunus laurocerasus*, *Bl.*, on ash stump, *S.*
Poria mollusca, on sycamore log, *Bl.*; [1]versipora, *Bl.*; [1]viridans, *C.*, on very rotten sycamore, *Bl.*
Solenia candida, *Bl.*, on very rotten wood, *B.*
Stereum ?chailletii, on bark of *Rhododendron*, *Bl.*; hirsutum, *B.*, *Bl.*, *D.*, on sycamore log, *Bl.*; purpureum, on oak, *D.*; sanguinolentum, on birch, *D.*, on beech stump, *S.*; spadiceum, on bark of larch, *Bl.*
Tomentella cinerascens (Karst.) v. H. & L., on base of living *Hippophae* trunk, *B.*; fusca (Pers. ex Fr.) Schröt., on waterlogged stick of birch, *D.*
Trametes gibbosa on dead wood, *B.*; rubescens, *D.*

Agaricales

Amanita spissa, *D.*
Amanitopsis fulva, *D.*
Bolbitius vitellinus, under conifers, *D.*
Boletus edulis, *D.*; erythropus, in mixed woodland, *D.*; ?leoninus Pers., *D.*; parasiticus, *D.*
Cantharellus cibarius, *D.*
Clitocybe aurantiaca, *C.*, *D.*; infundibuliformis, *B.*
Collybia fusipes, *B.*; radicata, *B.*
Coprinus erythrocephalus Lév., *S.*; hiascens, *Bl.*, *C.*, *S.*; lagopus, *C.*; plicatilis, *C.*, *D.*
Cortinarius helvelloides, *D.*; armillatus, *D.*
Crepidotus mollis, *L.*, *S.*; sphaerosporus, *B.*; variabilis *sensu stricto*, *D.*
Crinipellis stipitarius, *B.*
Flammula penetrans, *D.*
Galera appendiculata, *C.*; coprophila, Kühner, *Cheddar*; lactea, *B.*; rickenii J. Schaef. (=G. siliginea auct. non Fr.), *D.*
Gomphidius rutilus, *C.*
Hygrophorus langei Kühner, *B.*
Hypholoma hydrophilum, *D.*; leucotephrum; velutinum, *Bl.*, *D.*
Inocybe napipes, *D.*; †tabacina Furrer, *Bl.*

Laccaria laccata, *D.*
Lactarius camphoratus, *D.*; mitissimus, *D.*; quietus, *D.*; obscuratus, *D.*
Marasmius androsaceus, *D.*; confluens, *Bl.*; dryophilus, *Bl.*, *D.*; epiphyllus, *C.*, *D.*, *L.*, *S.*; fusipes, *D.*; ramealis, *D.*, *S.*, on oak wood and dead *Rubus* stems, *Symonds Yat*; rotula, *Bl.*, *C.*, *L.*, *S.*
Mycena acicula, *B.*, *Bl.*, *C.*, *L.*; amicta, *D.*; ammoniaca, *C.*, *L.*; citrino-marginata Gill., *C.*; clavicularis Fr., *C.*; epichloe Kühner, on leaves of monocots. in water, *B.*; floccipes, *L.*; galericulata, *Bl.*, *D.*, *L.*, *S.*; galopus, *Bl.*, *C.*, *D.*; rorida, *Bl.*, *C.*, *S.*; sanguinolenta, *C.*, *D.*; speirea, *C.*, *L.*; stylobates, *D.*; tenerrima, *Bl.*, *C.*, *D.*, *L.*, *S.*; vitilis (non Kühner), *C.*, *L.*
Naucoria centunculus, *Bl.*, *C.*; †muricata *var.* gracilis Quél., *L.*
Nolanea cetrata, *D.*
Omphalia fibula, *D.*; gracilis, on twigs of *Hippophae*, *B.*; stellata, on twigs of *Hippophae*, *B.*
Panus stipticus, *D.*
Paxillus ?filamentosus Bres., *S.*; involutus, *D.*, *S.*
Pholiota mutabilis, *L.*; sphaleromorpha, *B.*
Pleurotus cornucopiae, *Bl.*, *L.*; ostreatus, *D.*
Pluteus cervinus, *C.*, *D.*; nanus, *S.*; *var.* lutescens, *C.*, *L.*, *S.*; phlebophorus, *B.*; salicinus *var.* beryllus (Pers.) Fr., on wood, *C.*; villosus (Bull.) Quél., under angiosperms, *D.*
Psathyrella atomata, *D.*; bifrons, *D.*; disseminata, *Bl.*; conopilea, *Bl.*, egenula (B. & Br.) Moser, *Bl.*; †orbitarum Romagn.; †pseudo-gracilis Romagn., *Bl.*
Psilocybe coprophila, *D.*; foenisecii, *D.*
[2]†Rhodophyllus (Eccilia) leptonipes Romagn., *B.*
Russula atropurpurea, *D.*; emetica, *D.*; laurocerasi, *D.*; lepida, *D.*; nigricans, *D.*; ochroleuca, *D.*; venosa, *D.*; vesca, *D.*
Stropharia semi-globata, *D.*
Tricholoma aggregatum, *S.*; rutilans, *D.*
Volvaria volvacea, *Bl.*

GASTEROMYCETES

[3]Cyathus striatus, *C.*
[3]Geastrum nanum, *B.*
Lycoperdon pyriforme, *S.*
Phallus impudicus, *D.*
[3]Scleroderma aurantium, *D.*

FUNGUS HYPOGEUS

ASCOMYCETES

Tuber nitidum, *W.*

FUNGI IMPERFECTI

COELOMYCETES

Actinonema rosae, *S.*
Hendersonia vagans, on *Fraxinus*, *C.*
Libertella faginea, *F.*
Phyllosticta hederaecola, *Bl.*; buxina, *Bl.*, *C.*
Septogloeum ulmi, *C.*
Septoria apii, *B.*; cornicola, *C.*; epilobii, *C.*; rubi, *B.*; violae, *C.*

HYPHOMYCETES

Acrimoniella atra.
Arthrobotrys sp., *Bl.* (river).
Asterosporium hoffmanni Kunz, on rotten beech bark, *D.*
Beauveria bassiana, on insect on rotten wood, *D.*
Bispora monilioides, *Bl.*
Botryosporium pulchrum, *C.*
Cercospora mercurialis, *Bl.*, on *Mercurialis perennis*, *C.*
Cladosporium herbarum, on *Corylus*, *C.*
Clavariopsis tenuis De Wild., *River Yeo.*
Constantinella tillettei (Desm.) Mason & Hughes, on oak chips, *C.*

Dendryphion laxum Berk. & Br., on *Urtica, Symonds Yat.*
Didymaria didyma, on *Ranunculus repens, Bl., C., S.*
Epicoccum purpurascens.
Flagellospora curvula Ingold.
Helicomyces tenuis Speg., *Bl.* (river).
Isaria farinosa, *Bl.*, on insect, *D.*
Oidium alphitoides Griff. & Maub., *C., D., S.*; aureum Fr., on rotten wood, *C.*; euonymi-japonicae (Archang.) Sacc., *B.*
Ovularia obliqua, *C.*; primulana, *S.*; veronicae, *S.*
Penicillum brevicompactum, on *Clitocybe aurantiaca* and *Polyporus betulinus, C.*, on *Boletus edulis, D.*
Periconia byssoides, on *Urtica dioica, C., Symonds Yat.*
Polythrincium trifolii, on *Trifolium repens, B.*
Ramularia arvensis, *S.*, on *Potentilla anserina, B.*; lysimachiarum, on *Lysimachia nummularia, D.*; sambucina, *B.*; scophulariae, on *Scrophularia nodosa, C.*; senecionis, *Bl.*; taraxaci, *Bl.*; urticae, *Bl., D.*; valerianae, on *Valeriana officinalis, D.*

AUTUMN DAY FORAYS, 1955

As on previous occasions, we were given permission by a number of other Societies to advertise their own Day Forays in our programme. I would again like to thank the Societies concerned for welcoming any of our members who attended their meetings. We are always pleased to see members of other Societies on our own Day Forays.

Home Counties Day Forays organized by the British Mycological Society were held as follows: 24 *September*, Windsor Forest; 2 *October*, Juniper Hall, near Dorking; 9 *October*, Aldbury, near Tring; 15 *October*, Ruislip (Middlesex); 23 *October*, Burnham Beeches (Joint Foray with Middle Thames Natural History Society).

As might be expected from the very dry weather, these Day Forays were not very rewarding. However, at the Aldbury foray, a new British record was made in the agaric *Russula parazurea* J. Schaeff.

In other provincial areas, and in Scotland, the following Day Forays were advertised in our programme: 17 *September*, Glasgow and Andersonian Natural History and Microscopical Society at Dundonald; 25 *September*, Sorby Natural History Society; 1 *October*, Glasgow and Andersonian Natural History and Microscopical Society at Ashgrove; 8 *October*, Edinburgh Natural History Society at Gifford; Glasgow and Andersonian Natural History and Microscopical Society at Cumbernauld House; 9 *October*, Lickey Hills, Birmingham; 15 *October*, Sheffield University Botany Department at Ecclesall Wood and Limb Valley; Glasgow and Andersonian Natural History and Microscopical Society at Mugdock Reservoir; 16 *October*, Sheffield University Botany Department, at Longshaw Pastures: 22 *October*, Lincolnshire Naturalists' Union.

F. B. HORA (*Foray Secretary*)
The University
Reading, Berks

REVIEWS

Biology of Root-infecting Fungi. By S. D. GARRETT. (Cambridge University Press, 1956.) Pp. xi + 293, 1 text-figure. Price: 30*s*.

From time to time Dr Garrett has published illuminating summaries of the increasing knowledge of the biology of soil fungi, to which he has himself contributed in such large measure. The present book far out-distances these in scope and in detail of information.

The book begins with a masterly survey of the ecological groups of soil fungi; a grouping that owes much to Dr Garrett's own studies. Two chapters are then devoted to the parasitic growth habit of particular types of pathogenic root-inhabiting fungi and a third to that of mycorrhizal fungi. The effects of the microfloras of the root surface and rhizosphere on root infection are then considered. This chapter summarizes and integrates a great deal of previously scattered information. Chapters on saprophytic behaviour of root-infecting fungi and on their dormant phases are followed by a consideration of the epidemiology of root diseases and of their control. This is indeed a comprehensive survey which has been made possible only by Dr Garrett's wide knowledge of the subject as a whole, and his more intimate acquaintance with many particular aspects through his extensive researches on root-infecting fungi. The full bibliography greatly enhances the value of the book.

The soil is a complex and heterogeneous substrate and the biology of soil organisms is not only itself complex but its study is of great difficulty. It is not surprising, therefore, that no branch of biology has suffered more from too hasty conclusions and misinterpretations of data. Dr Garrett goes far towards clearing away these false conclusions and restores the subject to sound foundations. Anyone commencing research on a soil organism may well be saved much time and wasted effort by careful reading of this book.

In dedicating the book to Prof. W. Brown, F.R.S., Dr Garrett has acknowledged the great debt that all Prof. Brown's former students owe to him for his training and, in particular, to the example of his exceptional vision and clarity of thought. Dr Garrett, however, has shown himself a worthy pupil and has for many years made his own distinctive contribution, not only to plant pathology, but to what may be termed the Philosophy of Mycology, that is the understanding of the fungus as a living, competitive organism.

This is not a book to skim and put aside but one to read and re-read, since every paragraph is packed with information and ideas and the whole mass of data is woven together to make a coherent story for the first time, so that the reader finds much food for thought and further speculation.

The book is singularly free from errors and the printing and binding are of high standard. Both author and publishers are to be congratulated on such a valuable publication.

LILIAN E. HAWKER

Perspectives and Horizons in Microbiology. A Symposium edited by S. A. WAKSMAN (New Brunswick: Rutgers University Press, 1955). Pp. x + 220. Price: U.S. $3.50.

This book contains thirteen papers presented at a symposium held to mark the opening of the new Institute of Microbiology at Rutgers University in 1954. All the papers are well-written and informative. Some, and notably that by van Niel on 'The Microbe as a Whole', are remarkable for their stimulating breadth of view. No-one can read this book without enjoyment and profit. At the same time it is doubtful whether it completely justifies its title. The word microbiology means different things to different people, but it should be something more than a name for the more fashionable trends in bacteriology. Most of the papers in the symposium are about bacteria and most have a pronounced biochemical flavour. This bias, which probably reflects the present

disposition of research effort, gives point to van Niel's belief that science would benefit if 'tentative and often unrewarding probings into the behaviour of micro-organisms on a level now beyond the scope of biochemical and biophysical experimentation were not merely tolerated but encouraged'. Only two papers specifically deal with fungi. In one, J. W. Foster provides a wealth of evidence for the unique character of much of the metabolic activity of fungi, which nevertheless can be related in a general sense to the metabolic activities of other kinds of micro-organisms. In the other, D. H. Peterson gives a much-needed summary of a newly developed technique—the use of fungi for carrying out reactions in steroid chemistry difficult or impossible by more conventional methods of the chemical laboratory. P. W. BRIAN

Phytopathologie des pays chauds, Tome III. By L. ROGER. (*Encyclopédie Mycologique*, t. XIX. Paris: Paul Lechevalier, 1954.) Pp. 900, 7 text-figs. Price: French Fr. 10,000.

This third volume completes Dr Roger's compendious handbook of plant pathology for use in the tropics. The first part of the volume finishes the etiological survey of plant diseases which forms the backbone of the whole work, dealing with algae, lichens and mosses, bacteria, flowering plants and viruses as agents of disease. The virus section contains a general account of the history and nature of plant viruses occupying 80 pages. The second part of this volume surveys diseases of tropical crops arranged under the usual economic crop classification. The last 200 pages of the volume are occupied by a glossary, by an analytical index, and by excellent alphabetical subject, pathogen and host indexes which should greatly ease the reader's task of consulting the three volumes.

The work as a whole, and this volume is no exception, suffers from lack of pictures of disease symptoms—a result of the destruction of Dr Roger's collection of photographs during the war. Again, while many authors are cited in the text by name, the work contains no literature references and it cannot therefore be used as a source book. Dr Roger's treatise stands on its own as a work of monumental proportions. P. H. G.

Guide practique de mycologie médicale. By JEAN COUDERT. (Paris: Masson et Cie, 1955.) Pp. xiv + 364, 8 plates. Price: Wrappers, French Fr. 5200; Bound, French Fr. 6000.

El diagnóstico micológico de laboratorio. By RICARDO C. ZAPATER. (Buenos Aires: El Ateneo, 1956.) Pp. xiii + 161, 54 figures. Price: Pesos Argentinos 65 (approx. U.S. $2.00).

Both these books are designed to aid the diagnosis of mycotic infections of man and neither will satisfy every reader. Coudert's handsomely produced volume will, because the most ambitious, certainly prove to be the most generally disappointing. It begins with a tabulated summary of the principal mycoses of man, a useful section on mycological techniques, and keys to mycoses and to species of pathogenic fungi, while the bulk of the book (pp. 97–343) is devoted to standardized descriptions of fungi pathogenic for man and higher animals. For each species the clinical aspects of the condition induced are noted together with macro- and microscopic features of the pathogen, etc. There are no illustrations of individual species and as the compilation is, by present-day standards, uncritical in that within groups such as the Actinomycetes and the Dermatophytes and genera such as *Madurella* numbers of very doubtful species are accepted, an unwary inquirer is very likely to be misled. Also, double citation of authorities is not employed and it is possible that several new combinations have been perpetrated.

Perhaps the most helpful, and certainly the most attractive, feature of Zapater's little manual is the series of informative illustrations. Many of these are original and almost without exception the reproduction is good. The text includes a key to the principal pathogenic fungi, recipes for the commoner mycological culture media, and brief, often rather too brief, accounts of the chief mycotic infections of man and animals and the causal agents, together with short bibliographies. This useful addition to the South American literature is not without interest elsewhere. G. C. AINSWORTH